

# Parental Functions During Conjugation in *Escherichia coli* K-12

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## INTRODUCTION

A partial understanding of the mechanisms operative during conjugation between Hfr donors and F<sup>-</sup> recipients of *Escherichia coli* K-12 has been achieved as a consequence of studies performed in numerous laboratories. Jacob and Wollman (39) and Hayes (35) have evaluated and summarized many of the earlier studies. Recent efforts to further elucidate the nature of events during conjugation have been primarily concerned with testing the mechanisms for chromosome transfer proposed in 1963 by Bouck and Adelberg (8) and by Jacob, Brenner, and Cuzin (38). Bouck and Adelberg proposed that Hfr cells had to complete their present cycle of vegetative chromosome replication prior to initiating transfer of their chromosomes without

further replication. Jacob, Brenner, and Cuzin postulated that replication of the donor chromosome during transfer was obligatory and acted as the driving force for transfer. They also proposed that this chromosome replication during transfer was initiated following a contact stimulus received from the F<sup>-</sup> parent, was controlled by genes present in the integrated fertility factor F, and was independent of any system for controlling chromosome replication during vegetative growth. Adelberg and Pittard (2) have included in their recent review a detailed discussion of these two proposed mechanisms for chromosome transfer. Most investigators who have attempted to differentiate between these two models have obtained results which are at variance with the predictions of the Bouck-Adelberg scheme but in accord with the expectations of the Jacob-Brenner-Cuzin hypothesis (4, 6, 24, 25, 33, 34, 36, 49).

Jacob, Brenner, and Cuzin (38) reiterated, as

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part of their model, Fisher's (28) conclusion that the  $F^-$  parent is passive during chromosome transfer. Bonhoeffer (7), Freifelder (30), and the present authors (20; Curtiss, Mays, and Stallions, *Bacteriol. Proc.*, p. 55, 1967) have obtained data which suggest that the  $F^-$  parent may perform a very active role during chromosome transfer. This manuscript contains evidence we have used in postulating a mechanism for chromosome mobilization and transfer which requires the active participation of both mating partners.

#### EXPERIMENTAL APPROACH

In the experiments reported in this paper, we used donor and recipient mutants unable to utilize various energy sources or to synthesize various metabolites. We conducted matings in the presence or absence of these substances as a means of controlling energy metabolism or macromolecular syntheses, or both, in one or both parents. These experiments were done to determine which parent must be capable of performing which biosynthetic activities during the initiation and continuance of chromosome transfer. Before discussing the experiments conducted and the interpretation of the results obtained, we should mention certain features of our experimental approach to understanding the functions of each parent during bacterial conjugation.

First, we subdivided bacterial conjugation into five steps and *attempted* to study them experimentally one at a time under conditions which are optimal for all steps other than the one being studied. The five steps are (i) formation of specific pairs between donor and recipient cells, (ii) conversion of specific pairs to effective pairs or conjugation tube formation, (iii) chromosome mobilization or the initial events in the donor cell for the conversion of a circular chromosome into a chromosome capable of being sequentially transferred, (iv) chromosome transfer, and (v) integration of the transferred donor chromosome into the recipient chromosome to produce recombinants. Brinton and his collaborators (9-11) have shown that F pili on donor cells are absolutely necessary for specific pair formation. We (R. Curtiss and L. G. Caro, *Bacteriol. Proc.*, p. 27, 1966; *manuscript in preparation*) have confirmed this conclusion and have discovered growth conditions for donor parents which maximize the mean number and length of F pili per cell. We have also shown that specific pair formation occurs at unaltered frequencies in the absence of energy metabolism in either parent (22). Therefore, all of the experiments conducted during the past several years which are reported in this manuscript were done under conditions in which essentially all donor cells form specific

pairs with one or more recipient cells. Since the exact nature of conjugation tube formation is probably not understood (Curtiss and Caro, *in preparation*), it is possible that the metabolic activities required during the inception of mating are needed for conjugation tube formation or chromosome mobilization, or for both.

The second feature of our experimental approach to understanding bacterial conjugation is the use of recombinant formation in the recipient as an indication of chromosome transfer in most experiments. It is preferable in some instances to use indicators of chromosome transfer which do not require integration of the transferred material into the recipient chromosome, and this was done when possible. However, such indicators of transfer as enzyme synthesis, episome replication, and zygotic induction of a prophage all require energy metabolism and macromolecular syntheses in the recipient for detection. The initial recombination event(s) both in phage (56, 57) and in bacteria (19) probably can occur in the absence of energy metabolism. Therefore, we believe that recombinant production is the best genetic indicator of chromosome transfer in studies in which energy metabolism or macromolecular syntheses are being inhibited in the recipient parent.

The third feature of our experimental approach is the use of isogenic donor and recipient strains, derived in our laboratory from the prototrophic  $F^+$  strain W1485 (43). We were prompted to do this by the realization that most donor and recipient sublines of *E. coli* K-12 have not shared the same ancestor for over 20 years. Thus, by estimating the number of cell division cycles needed for the introduction of each individual mutation and for routine transfers during these 20 years, we estimate that the existing representatives of these sublines are separated by about 10,000 cell division cycles of growth. This is roughly equivalent to about one-quarter million years in human evolutionary time.

#### MATERIALS AND METHODS

##### *Media*

The formulas for minimal liquid and minimal agar media have been described (18). The minimal mating medium contained the same total concentration of salts as minimal liquid medium, except that  $K_2HPO_4$  and  $KH_2PO_4$  were added at 4.9 and 6.3 g/liter, respectively, to achieve a final pH of 6.3. Glucose was used as the carbon source unless otherwise indicated. All carbon sources were used at a final concentration of 5.0 g/liter. Difco Casamino Acids were used as a supplement to minimal medium in some experiments. There was no detectable difference between use of solutions of Casamino Acids which

had been treated with activated charcoal and those which had not. Supplements were purchased from Calbiochem (Los Angeles, Calif.) and were used at the following concentrations (in  $\mu\text{g/ml}$ ): L-alanine, 100; L-aspartic acid, 100; L-asparagine, 100; L-arginine HCl, 22; L-cysteine HCl, 22; glycine, 100; L-glutamic acid, 100; L-glutamine, 100; L-histidine HCl, 22; L-isoleucine, 20; L-leucine, 20; L-lysine HCl, 88; L-methionine, 10; L-phenylalanine, 20; L-proline, 30; DL-serine, 100; DL-threonine, 80; L-tryptophan, 20; L-tyrosine, 20; L-valine, 20; adenine, 40; uracil, 40; thymidine, 4; thymine, 4; thiamine HCl, 2; and streptomycin sulfate, 200.

Difco Penassay broth and agar, L broth and L agar (44), and EMB agar (19) were used as complex media. Buffered saline with gelatin (18) was used in some experiments.

#### *Bacterial Strains*

The bacterial strains are listed in Table 1 with their derivation. All mutations either were of spontaneous occurrence or were induced by low doses of ultraviolet irradiation or by low concentrations of nitrous acid. The enrichment procedures for isolation of auxotrophic mutants and Hfr donors have been described (5, 21). All strains were frozen soon after isolation for long-term storage. Working stocks were maintained on Penassay agar slants at 4 C.

All strains used to determine the requirements for energy metabolism and macromolecular syntheses during chromosome mobilization and transfer possess mutations which are not leaky and which either do not revert or revert at low frequency. Strains  $\chi$  584 and  $\chi$  724 have *thy*<sup>-</sup> mutations which do not revert and are not temperature sensitive. These strains, isolated by enrichment with aminopterin, will grow slowly with 1  $\mu\text{g}$  of thymine/ml and at optimal rates with 4  $\mu\text{g}$  of thymine/ml. When these strains are resuspended in thymine-deficient medium after being washed once, there is a 5 to 10% increase in deoxyribonucleic acid (DNA) which occurs during the first 20 min after removal of thymine, as measured by the diphenylamine reaction. Thereafter, there is no detectable net synthesis of DNA.

#### *Mating Procedures*

During the course of these studies, the procedures for conducting matings have been markedly improved. The following format has been used during the past 2 or 3 years, and exceptions to these procedures are listed in the table footnotes and figure legends. Bacteria are grown for 8 to 10 generations in tubes (25  $\times$  200 mm) containing L broth or containing minimal medium supplemented with 0.5% (w/v) Casamino Acids.

Donor strains are grown without aeration to achieve maximal numbers and lengths of F pili per cell (Curtiss and Caro, *in preparation*), and recipient strains are grown with aeration at 37 C. Sedimentation, washing, resuspension, and starvation are all conducted at 37 C to minimize any temperature shocks. Great care is taken with donor cultures to avoid breakage of F pili. The matings are conducted in 10-ml volumes in 125-ml microfernbach flasks (Bellco Glass, Inc., Vineland, N.J.) which are immersed up to within 5 to 10 mm of the metal caps in a water bath maintained at 37 C. The donor-to-recipient cell ratio is usually between 1:10 and 1:20. The total bacterial density is no more than  $2 \times 10^8/\text{ml}$  at the commencement of mating to avoid problems of oxygen depletion toward the end of matings of long duration. Interruption of mating is accomplished by diluting samples of the mating mixture into ultraviolet (UV)-irradiated ( $2.5 \times 10^3$  ergs/mm<sup>2</sup>), purified T6 bacteriophage at  $2 \times 10^{10}$  particles/ml, final concentration. This suspension is then immediately agitated on a vortex mixer for 15 sec to separate mating partners. Following 15 to 16 min at 37 C, antiserum to T6, prepared by injecting rabbits with purified T6, is used to neutralize any unadsorbed T6. After another 15 to 16 min at 37 C, appropriate dilutions are plated on streptomycin-containing media selective for the desired class of recombinants.

The integration of transferred donor genetic material into the recipient chromosome to yield recombinants requires a complex series of events. Thus, it is essential to provide conditions which will allow the F<sup>-</sup> parent to perform all necessary metabolic functions associated with integration to obtain maximal recombinant yields. Therefore, the minimal medium used for the T6 treatment and all subsequent dilutions was appropriately supplemented to provide glucose at a final concentration of 0.5% (w/v) and all other metabolites required by the F<sup>-</sup> parent. For matings in L broth, the minimal medium used as diluent contained 10% L broth (v/v).

#### *Controls*

Since Hfr strains differ with respect to the stability of F integration (1, 12), we routinely determined the frequency of Hfr versus "non-Hfr" donors in the cultures used for matings by using the cross streak methods outlined by Berg and Curtiss (5). Reisolation of pure Hfr cultures was sometimes necessary. Hfr OR1 (Curtiss, Bacteriol. Proc., p. 30, 1964) is very unstable, and cultures descended from single colonies contain between 60 and 90% Hfr cells. We showed, however, by analyzing the mating type of recombinants inheriting a marker transferred early by

Hfr OR1, that the "F<sup>+</sup> type" donors in the Hfr OR1 population were not contributing more than a few per cent of the recombinants formed. [The mating type of recombinants was deter-

mined by cross streaking log-phase recombinant cultures across a donor-specific phage on EMB agar containing 0.1% glucose (5).] The recombinant frequencies in matings with Hfr OR1 were

TABLE 1. *Bacterial strains*<sup>a</sup>

Strain no.	Mating type	Relevant genotype <sup>b</sup>	Derivation
x 18	F <sup>+</sup>	prototroph $\lambda^- str^r$	x 15 <sup>c</sup>
x 57	Hfr H	$thi^- \lambda^- T6^s str^s$	3000 <sup>d</sup>
x 91	Hfr H	$thi^- \lambda^+ T6^s str^s malA^+ malB^+$	x 57
x 96	Hfr H	$thi^- \lambda^+ T6^s str^s malA^- malB^-$	x 91
x 99	F <sup>-</sup>	$thr^- leu^- thi^- \lambda^- T6^s str^r malA^+ malB^+$	x 12 <sup>d</sup>
x 100	F <sup>-</sup>	$thr^- leu^- thi^- \lambda^- T6^s str^r malA^- malB^-$	x 12 <sup>d</sup>
x 137	F <sup>-</sup>	$thr^- leu^- proA-B^- thi^- \lambda^- T6^r str^r$	C600 <sup>e, d</sup>
x 225	Hfr OR1	prototroph $lacZ^- \lambda^- T6^s str^s$	x 15 <sup>e, e</sup>
x 235	Hfr Cav	$met^- \lambda^- T6^s str^s$	x 11 <sup>e</sup>
x 277	F <sup>-</sup>	$thr^- leu^- proC^- thi^- \lambda^- T6^r str^r$	C600 <sup>e, d</sup>
x 278	F <sup>-</sup>	$thr^- leu^- proB^- thi^- \lambda^- T6^r str^r$	C600 <sup>e, d</sup>
x 289	F <sup>-</sup>	prototroph $\lambda^- T6^s str^s$	x 15 <sup>e, f</sup>
x 313	Hfr P4X6	$met^- thy^- \lambda^+ T6^s str^s$	AB2383 <sup>g</sup>
x 314	F'	$lac^- thi^- \lambda^- T6^s str^s/F-lac^+$	AB785 = 200PS <sup>h</sup>
x 354	F <sup>-</sup>	$proB-lac^- \lambda^- T6^s str^s$	x 289
x 435	Hfr OR6	prototroph $\lambda^- T6^s str^s$	x 42 <sup>e</sup>
x 436	Hfr OR7	$his^- \lambda^- T6^s str^s$	x 42 <sup>e</sup>
x 493	Hfr OR11	prototroph $\lambda^- T6^s str^s$	x 15 <sup>e, i</sup>
x 503	Hfr OR21	prototroph $xyl^+ \lambda^- T6^s str^s$	x 15 <sup>e, j</sup>
x 540	F <sup>-</sup>	$purE^- pyr^- his^- xyl^- \lambda^- T6^r str^r$	x 289
x 545	F <sup>-</sup>	$proB-lac^- \lambda^- T6^s str^r$	x 354
x 584	Hfr OR41	$proB-lac^- thy^- \lambda^- T6^s str^s$	x 354
x 646	F'ORF-206	$lacY^- T6^s \lambda^- str^s/F-lac^+ proB^+ proA^+$	x 535 <sup>i</sup>
x 647	F'ORF-207	$lacY^- T6^s \lambda^- str^s/F-lac^+$	x 629 <sup>i</sup>
x 680	F <sup>-</sup>	$thr^+ leu^- proA^- thi^- \lambda^- T6^r str^r$	C600 <sup>e, d</sup>
x 696	F <sup>-</sup>	$proB^- leu^- arg^- \lambda^- T6^r str^r$	x 289
x 705	F <sup>-</sup>	$leu^- arg^- \lambda^- T6^r str^r$	x 696
x 708	F <sup>-</sup>	$lac^- proB^- leu^- arg^- \lambda^- T6^r str^r$	x 696
x 710	F <sup>-</sup>	$proA^- leu^- arg^- \lambda^- T6^r str^r$	x 705
x 711	F <sup>-</sup>	$proA-B^- leu^- arg^- \lambda^- T6^r str^r$	x 705
x 723	F <sup>-</sup>	$thr^- purE^- pyr^- his^- xyl^- \lambda^- T6^r str^r$	x 540
x 724	F <sup>-</sup>	$thr^- purE^- pyr^- his^- xyl^- thy^- \lambda^- T6^r str^r$	x 723
x 733	F <sup>-</sup>	$lacZ-I^- leu^- arg^- \lambda^- T6^r str^r$	x 705 × P1kc (1485-1 <sup>k</sup> )
x 775	F <sup>-</sup>	$purE^- pyr^- trp^- his^- xyl^- malA^- \lambda^- T6^r str^r$	x 540
x 784	F <sup>-</sup>	$proB-lac^- leu^- arg^- \lambda^- T6^r str^r$	x 705 × P1kc (x 354)
x 800	F <sup>-</sup>	$purE^- pyr^- trp^- his^- xyl^+ malA^- \lambda^- T6^r str^r$	x 775 × P1kc (x 289)
x 801	Hfr OR21	prototroph $xyl^- \lambda^- T6^s str^s$	x 503
x 820	F <sup>-</sup>	$thr^- proC^- purE^- pyr^- his^- xyl^- \lambda^- T6^r str^r$	x 723
x 821	F <sup>-</sup>	$thr^- proA^- purE^- pyr^- his^- xyl^- \lambda^- T6^r str^r$	x 723
x 927	F' KLF-1	$thr^- leu^- pro^- met^- thi^- T6^r str^s/F-thr^+ leu^+$	CB069 <sup>l</sup>

<sup>a</sup> The abbreviations and nomenclature used follow the proposal of Demerec et al. (27) with the exceptions noted by Curtiss (19).

<sup>b</sup> All mutations conferring auxotrophic requirements are listed, but some mutations conferring resistance to drugs and phage and inability to utilize carbon sources have been omitted for sake of brevity.

<sup>c</sup> Curtiss (17).

<sup>d</sup> Curtiss (18).

<sup>e</sup> Curtiss (19).

<sup>f</sup> Curtiss et al. (21).

<sup>g</sup> From K. Szende by way of N. Schwartz.

<sup>h</sup> From F. Jacob by way of E. Adelberg and N. Schwartz.

<sup>i</sup> Berg and Curtiss (5).

<sup>j</sup> Curtiss and Stallions (22).

<sup>k</sup> Schwartz (52).

<sup>l</sup> From B. Low by way of C. M. Berg.

therefore calculated by using the actual titer of Hfr cells.

In all experiments in which one or both parents were starved for a carbon source or metabolite, the mutation causing the inability to utilize or synthesize the compound was checked for reversion by plating undiluted samples of the parent culture on appropriate selective medium. The parent cultures were also checked for inability to grow on the media used for recombinant selections.

In crosses between nonisogenic parents, the colonies which arise on selective medium are not always due to the presence of haploid recombinants (18). Therefore, we frequently picked and analyzed colonies formed on selective media to determine haploidy and purity of recombinant type. This was usually done the first time a particular mating was performed. Data from matings in which problems of partial diploidy, impurity of recombinant clones, or distorted linkage were observed are not included in this manuscript. These problems are of infrequent occurrence in matings between isogenic parents.

### Reproducibility of Experiments

The experiments cited in this communication were initiated in August 1961 and completed in April 1968. Each type of experiment was performed by two or more of the authors and with a great variety of bacterial strains. Most of the data obtained from matings with nonisogenic parents have been omitted. The experimental results obtained with different substrains of *E. coli* K-12 and by different authors were essentially similar for the same type of experiment.

## RESULTS

### *Effects of Inhibiting Energy Metabolism in One or Both Parents Before and During Conjugation*

*Effects of growth conditions and starvation.* Fisher (28) proved that zygote formation was an endergonic process requiring the aerobic utilization of an energy-yielding carbohydrate. He concluded from results obtained in experiments with starved or unstarved parents that only the Hfr donor required an energy source during chromosome transfer. The first three lines of Table 2

TABLE 2. *Effects of aeration during growth and starvation on recombinant production by Hfr and F<sup>-</sup> parents grown in broth<sup>a</sup>*

Growth conditions of		3-hr starvation of		Glucose present during mating	Frequency of <i>thr<sup>+</sup> leu<sup>+</sup> str<sup>r</sup></i> recombinants	
Hfr parent	F <sup>-</sup> parent	Hfr parent	F <sup>-</sup> parent		Actual <sup>b</sup>	Relative
(1) Aerated	Aerated	No	No	Yes	$8.3 \times 10^{-2}$	100
				No	$5.1 \times 10^{-2}$	61
(2) Aerated	Aerated	Yes	No	Yes	$1.9 \times 10^{-3}$	2.3 (100)
				No	$6.1 \times 10^{-4}$	0.73 (32)
(3) Aerated	Aerated	No	Yes	Yes	$1.1 \times 10^{-1}$	133 (100)
				No	$4.8 \times 10^{-2}$	58 (44)
(4) Static	Aerated	No	No	Yes	$9.8 \times 10^{-2}$	118 (100)
				No	$6.4 \times 10^{-2}$	77 (65)
(5) Aerated	Static	No	No	Yes	$1.5 \times 10^{-2}$	18 (100)
				No	$9.6 \times 10^{-3}$	12 (64)

<sup>a</sup>  $\times 57$  (Hfr H *thr<sup>+</sup> leu<sup>+</sup> str<sup>r</sup>*) and  $\times 99$  (F<sup>-</sup> *thr<sup>-</sup> leu<sup>-</sup> str<sup>r</sup>*) were grown for 3 hr in 20 ml of Penassay broth in 200  $\times$  25 mm tubes either with (aerated) or without (static) aeration to a cell density of about  $2 \times 10^8$ /ml. The bacteria were then sedimented, washed, and resuspended, either in buffered saline, if they were to be starved, or in minimal mating medium containing threonine, leucine, and thiamine, if they were to be mated. The 20-ml cultures starved in buffered saline with aeration were likewise sedimented and resuspended in minimal mating medium before mating. Glucose was added as indicated to the mating flask containing the F<sup>-</sup> parent 30 sec before adding the Hfr parent. Matings lasted for 40 min and were interrupted by dilution into minimal medium containing thiamine, glucose, and streptomycin, immediately followed by agitation on a vortex mixer for 60 sec. Appropriate dilutions were then plated on selective medium.

<sup>b</sup> Averages for three or more matings in which the inhibitory or stimulatory effects on recombinant yield were always in the same direction but differed in magnitude.

contain mean data from experiments similar to those performed by Fisher. It is evident that starvation of broth-grown Hfr cultures in buffered saline results in a marked decrease in recombinant yield, regardless of whether an energy source is present or absent during mating (line 2, Table 2). This nonrestorable loss of mating ability is primarily due to the fact that donor cells lose F pili at an exponential rate during starvation in buffered saline (Curtiss and Caro, *Bacteriol. Proc.*, p. 27, 1966; *manuscript in preparation*) and consequently lose the ability to form specific pairs with F<sup>-</sup> cells.

Starvation of broth-grown F<sup>-</sup> cells in buffered saline does not decrease the actual recombinant yield in matings conducted in the absence of an energy source (line 3 versus line 1, Table 2) as was noted by Fisher (28). However, starved F<sup>-</sup> cultures consistently yield higher recombinant frequencies than do unstarved F<sup>-</sup> cultures in matings conducted with an energy source present (line 3 versus line 1, Table 2). [This behavior was also observed for amino acid-requiring F<sup>-</sup> strains starved for a required amino acid (*see below*) and is in accord with the observation that stationary phase F<sup>-</sup> cells give higher recombinant frequencies than do log-phase F<sup>-</sup> cells.] Thus, to correct for the increased recombinant yield found in matings with starved F<sup>-</sup> cells conducted in the presence of glucose, it is necessary to

determine relative recombinant yields. When this is done, it is found that starvation of the F<sup>-</sup> parent prior to mating results in fewer recombinants (44%) than when the F<sup>-</sup> is not starved (61%). These effects of starvation on the F<sup>-</sup> parent are more pronounced when a distally transferred Hfr marker is used for recombinant selection (data not shown, but see experiments presented later in manuscript).

The data in line 4 (Table 2) demonstrate that Hfr donors grown without aeration yield more recombinants than do donors grown with aeration. The relative increase in recombinant yield for Hfr donors grown without aeration is more pronounced when the mating is of shorter duration than 40 min or when selection is for a distally transferred Hfr marker (data not shown). This behavior is probably accounted for by the observation that donor strains grown without aeration have a higher mean number of F pili per cell than do donor strains grown with aeration (Curtiss and Caro, *in preparation*). This would, therefore, allow a higher frequency of specific pair formation. The data in line 5 (Table 2) indicate that the ability of the F<sup>-</sup> parent to yield recombinants can be affected by the conditions of growth. Hfr and F<sup>-</sup> strains grown without aeration are less and more affected, respectively, by starvation in buffered saline than are

TABLE 3. Effects of starvation for an energy source prior to and during mating on recombinant production and zygotic induction by Hfr and F<sup>-</sup> parents grown in minimal medium<sup>a</sup>

Time (min) of starvation for glucose		Glucose present during mating	Actual frequency of <sup>b</sup>		Relative frequency of	
Hfr parent	F <sup>-</sup> parent		<i>thr<sup>+</sup> leu<sup>+</sup> str<sup>r</sup></i> recombinants	$\lambda$ infective centers	<i>thr<sup>+</sup> leu<sup>+</sup> str<sup>r</sup></i> recombinants	$\lambda$ infective centers
0	0	Yes	$1.4 \times 10^{-2}$	$1.2 \times 10^{-2}$	100	100
		No	$7.8 \times 10^{-3}$	$6.9 \times 10^{-3}$	56	58
0	80	Yes	$1.3 \times 10^{-2}$	$1.1 \times 10^{-2}$	93 (100)	92 (100)
		No	$2.1 \times 10^{-3}$	$1.2 \times 10^{-3}$	15 (16)	10 (11)
80	0	Yes	$3.6 \times 10^{-3}$	$3.8 \times 10^{-3}$	26 (100)	32 (100)
		No	$1.7 \times 10^{-3}$	$1.8 \times 10^{-3}$	12 (47)	15 (47)
80	80	Yes	$2.6 \times 10^{-3}$	$2.1 \times 10^{-3}$	19 (100)	18 (100)
		No	$2.6 \times 10^{-4}$	$1.5 \times 10^{-4}$	1.9 (10)	1.3 (7.1)

<sup>a</sup>  $\chi$  91 (Hfr H *thr<sup>+</sup> leu<sup>+</sup>  $\lambda^+$  str<sup>r</sup>*) and  $\chi$  100 (F<sup>-</sup> *thr<sup>-</sup> leu<sup>-</sup>  $\lambda^-$  str<sup>r</sup>  $\lambda^r$* ) were grown with aeration in appropriately supplemented minimal medium containing glucose to a cell density of about  $2 \times 10^8$ /ml. The bacteria were then sedimented, washed, and resuspended either in supplemented minimal medium lacking glucose, if they were to be starved for glucose, or in minimal mating medium containing threonine, leucine, and thiamine, if they were to be mated. The matings were terminated after 40 min. Other procedures are described in the footnote *a* of Table 2.  $\chi$  18, a streptomycin-resistant derivative of the F<sup>+</sup> strain  $\chi$  15, was used as the  $\lambda$  indicator strain.

<sup>b</sup> Average of two matings.

TABLE 4. Recombinant formation and zygotic induction in matings between *Hfr* and *F*<sup>-</sup> parents able or unable to utilize maltose<sup>a</sup>

Parents <sup>b</sup>		Energy source present during mating	Expt 1, 10-min starvation prior to mating				Expt 2, 40-min starvation prior to mating			
			Actual frequency of		Relative frequency of		Actual frequency of		Relative frequency of	
			<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>str</i> <sup>r</sup> recombinants	λ infective centers	<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>str</i> <sup>r</sup> recombinants	λ infective centers	<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>str</i> <sup>r</sup> recombinants	λ infective centers	<i>thr</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>str</i> <sup>r</sup> recombinants	λ infective centers
<i>Hfr</i>	<i>F</i> <sup>-</sup>	Glucose Maltose	6.3 × 10 <sup>-3</sup>	3.4 × 10 <sup>-3</sup>	100	54 (100)	1.2 × 10 <sup>-2</sup>	7.3 × 10 <sup>-3</sup>	100	61 (100)
			5.7 × 10 <sup>-3</sup>	3.6 × 10 <sup>-3</sup>	90	57 (106)	1.1 × 10 <sup>-2</sup>	7.1 × 10 <sup>-3</sup>	92	59 (97)
		Glucose Maltose	5.2 × 10 <sup>-3</sup>	2.4 × 10 <sup>-3</sup>	100	46 (100)	1.1 × 10 <sup>-2</sup>	7.1 × 10 <sup>-3</sup>	100	65 (100)
			4.0 × 10 <sup>-3</sup>	1.6 × 10 <sup>-3</sup>	77	31 (67)	5.4 × 10 <sup>-3</sup>	2.5 × 10 <sup>-3</sup>	49	23 (35)
		Glucose Maltose	7.8 × 10 <sup>-3</sup>	4.3 × 10 <sup>-3</sup>	100	55 (100)	1.2 × 10 <sup>-2</sup>	8.2 × 10 <sup>-3</sup>	100	68 (100)
			6.7 × 10 <sup>-3</sup>	3.1 × 10 <sup>-3</sup>	86	40 (72)	5.2 × 10 <sup>-3</sup>	3.7 × 10 <sup>-3</sup>	43	31 (45)
		Glucose Maltose	1.0 × 10 <sup>-2</sup>	5.2 × 10 <sup>-3</sup>	100	52 (100)	7.0 × 10 <sup>-3</sup>	5.1 × 10 <sup>-3</sup>	100	73 (100)
			4.0 × 10 <sup>-3</sup>	1.9 × 10 <sup>-3</sup>	40	19 (37)	1.2 × 10 <sup>-3</sup>	8.1 × 10 <sup>-4</sup>	17	12 (16)

<sup>a</sup> Bacteria were grown with aeration to log phase in supplemented minimal medium with glucose as an energy source. They were then sedimented, washed, and resuspended in minimal mating medium containing threonine, leucine, and thiamine but devoid of a principal carbon source. Glucose or maltose was added 5 min before commencement of mating to bacteria prestarved for 10 min (Expt 1) or 40 min (Expt 2) to remove endogenous energy reserves. Antiserum to λ at a dilution of 1:200 was included in the mating medium to neutralize free λ since one of the *F*<sup>-</sup> parents used was sensitive to λ. The matings were interrupted after 40 min. All other procedures were as described in the footnotes to Tables 2 and 3.

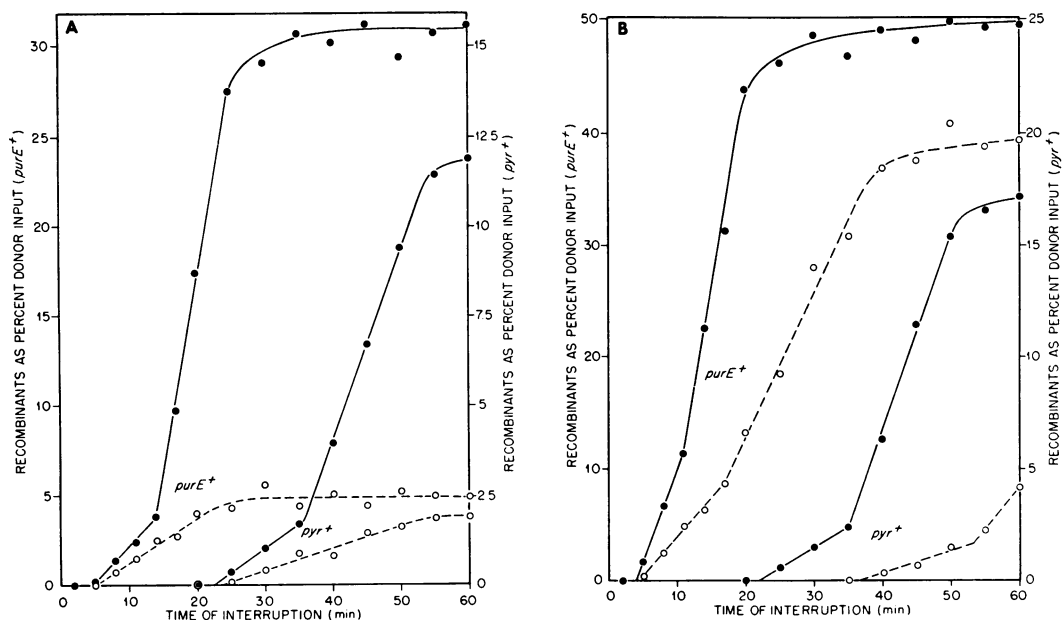
<sup>b</sup> The *Hfr* parents were derived from χ57 (*Hfr* H *thr*<sup>+</sup> *leu*<sup>+</sup> λ<sup>-</sup> *str*<sup>s</sup> *malA*, *B*<sup>+</sup>) and are λ<sup>+</sup> and either *malA*, *B*<sup>+</sup> (χ91) or *malA*, *B*<sup>-</sup> (χ96). The *F*<sup>-</sup> parents (*thr*<sup>-</sup> *leu*<sup>-</sup> λ<sup>-</sup> *str*<sup>r</sup>) used are likewise either *malA*, *B*<sup>+</sup> (χ99) or *malA*, *B*<sup>-</sup> (χ100).

the comparable strains grown with aeration (data not shown).

**Effects of starvation for an energy source prior to and during mating.** The data obtained in the experiments just presented indicated that starvation of either broth-grown parent for all carbon and nitrogen sources could cause a reduction in recombinant yield. To determine more accurately the nature of this effect, Hfr and  $F^-$  cultures were grown in minimal medium and then starved for glucose. The results of such experiments on recombinant production and zygotic induction are given in Table 3. It is apparent that the effects on the  $F^-$  parent of starvation for glucose prior to mating are reversed if glucose is present during mating. No such restoration of complete fertility returns to glucose-starved Hfr parents when they are mated in the presence of glucose (Table 3). This is probably due to the fact that F pili, which are lost during starvation, must be regrown before specific pair formation can occur with an ensuing transfer of genetic material.

The results presented in Table 3 indicate that active energy metabolism is probably required in both parents during conjugation. To reduce energy metabolism in one parent while allowing it to proceed normally in the other, Hfr and  $F^-$  mutants unable to utilize maltose were isolated. These strains, which were either able or unable to utilize maltose, were mated in all combinations in the presence of either glucose or maltose (Table 4). The results obtained clearly indicate that maximal frequencies of recombinant production and zygotic induction are only attained when both the Hfr and  $F^-$  parents are capable of unrestricted utilization of an energy-yielding carbohydrate.

**Function of energy metabolism in each parent during conjugation.** Interrupted mating experiments with the mutant strains used to collect the data in Table 4 were never very rewarding. First, the recombinant frequencies obtained in matings with cultures grown in minimal medium were always very low. Second, no plateau in



**FIG. 1.** Interrupted matings between Hfr and  $F^-$  parents able or unable to utilize xylose. (A) Hfr OR21  $purE^+$   $pyr^+$   $str^+$   $xyl^-$  (x 801) ×  $F^-$   $purE^-$   $pyr^-$   $str^+$   $xyl^+$  (x 800). (B) Hfr OR21  $purE^+$   $pyr^+$   $str^+$   $xyl^+$  (x 503) ×  $F^-$   $purE^-$   $pyr^-$   $str^+$   $xyl^-$  (x 775). The bacteria were grown in minimal medium containing adenine, uracil, tryptophan, histidine, glucose, and 0.1% Casamino Acids. The  $F^-$  parents were grown to about  $10^8$  cells/ml with aeration and the Hfr parents to about  $5 \times 10^8$  cells/ml without aeration to achieve maximal numbers of F pili per cells. The bacteria were sedimented, washed gently, and resuspended in prewarmed minimal mating medium containing adenine, uracil, tryptophan, and histidine, and the Hfr cultures were adjusted to a cell titer of about  $10^8$ /ml. The bacteria were starved for 30 min and then xylose (○) and glucose (●) were added to the appropriate cultures 10 min before matings commenced. The matings were interrupted by diluting into UV-irradiated T6 (suspended in minimal medium containing sufficient glucose to give a final glucose concentration of 0.5%) and immediately agitating with a vortex mixer for 15 sec. Unadsorbed T6 was neutralized with antiserum to T6. All manipulations were conducted at 37 C.

recombinant frequency was ever attained. In addition, Hfr H and the F<sup>-</sup> strains derived from W945 were not closely related. To circumvent these problems, further studies on the function of energy metabolism during conjugation employed isogenic Hfr and F<sup>-</sup> parents and made use of the fact that almost 100% specific pair formation could be achieved if the donor strains were grown without aeration in minimal medium containing Casamino Acids (Curtiss and Caro, *in preparation*).

Figure 1 illustrates the results obtained in interrupted matings, conducted in the presence of either glucose or xylose, between parents either able or unable to utilize xylose. Experiments like those presented in Table 4 were also done with these strains and similar results were obtained, although the recombinant frequencies were much higher than those listed in Table 4. The results obtained in interrupted matings between xylose-utilizing parents in the presence of either glucose or xylose were the same (data not shown). The results shown in Fig. 1A indicate that supply of a nonutilizable carbohydrate to the Hfr parent decreases the number of donor cells capable of initiating chromosome transfer without affecting the rate of chromosome transfer. These inferences are based on the observations that: (i) the times of first appearance of *purE*<sup>+</sup> and *pyr*<sup>+</sup> recombinants are the same in both matings, (ii) the length of mating time between the time of first appearance of either marker and the time of achieving a plateau frequency for that marker is the same in both matings, and (iii) the per cent inhibition in the frequency of *purE*<sup>+</sup> recombinants after 60 min of mating is about equal to the per cent inhibition in the frequency of *pyr*<sup>+</sup> recombinants.

The data shown in Fig. 1B indicate that supplying a nonutilizable energy source to the F<sup>-</sup> parent results in a decrease in the rate of chromosome transfer. The reasons for this interpretation are that: (i) the time of first appearance of *pyr*<sup>+</sup> recombinants is delayed 15 min for the mating in the presence of xylose, whereas the time of first appearance of *purE*<sup>+</sup> recombinants is the same in both matings; (ii) the length of mating time between the time of first appearance of *purE*<sup>+</sup> recombinants and the time of achieving a plateau frequency of *purE*<sup>+</sup> recombinants is much longer in the mating in the presence of xylose than in the mating in the presence of glucose; and (iii) the per cent inhibition in the frequency of recombinants for the distally transferred *pyr*<sup>+</sup> marker is greater after 60 min of mating than the per cent inhibition in the frequency of *purE*<sup>+</sup> recombinants.

Only some of the interrupted mating experi-

ments conducted to assess the parental requirements for energy metabolism during conjugation yielded results like those in Fig. 1. Therefore, certain comments about the difficulty of conducting these types of experiments and the validity of the conclusions drawn from them are necessary. Plateaus in the frequency of recombinants are only attained if all of the specific pairs which can form, are formed within the first few minutes of mating. This required that essentially all Hfr cells have F pili at the inception of starvation and that we not starve Hfr cultures for a great length of time prior to mating, which could cause a substantial loss of F pili. To accomplish this, we grew the donor cultures in the presence of 0.1% (w/v) Casamino Acids to high density without aeration and then diluted them after gentle washing for starvation in medium without Casamino Acids. The continued presence of Casamino Acids during mating provided the F<sup>-</sup> parent with enough energy so that there was little or no inhibition in recombinant yield or rate of chromosome transfer in experiments like the one presented in Fig. 1B. The total density of cells in the mating mixture and the ratio of donor cells to recipient cells also affected the results. The inhibitions on either parent were markedly reduced if the total cell density during mating was high (ca.  $5 \times 10^8$ /ml). Also, when the Hfr to F<sup>-</sup> cell ratio was 1:1 to 1:2, the degrees of inhibition achieved were greater in matings with the *xyl*<sup>-</sup> Hfr and smaller with the *xyl*<sup>-</sup> F<sup>-</sup> for matings conducted in the presence of xylose. Presumably, these effects are due to breakdown of xylose by the *xyl*<sup>+</sup> parent with utilization of the excreted breakdown products by the *xyl*<sup>-</sup> parent. We also obtained higher recombination frequencies and better overall results if the parents were grown on glucose prior to starvation rather than on glycerol or succinate. We demonstrated that the *xyl*<sup>+</sup> strains used could switch from utilization of glucose to xylose and vice versa with little or no lag.

To sum up our experience with the type of experiment presented in Fig. 1, it can be stated that we never observed changes in the time of first appearance of markers donated by Hfr parents unable to utilize the supplied carbon source but we frequently observed delays in the times of appearance of distally transferred markers in matings with F<sup>-</sup> parents unable to utilize the supplied carbon source. Therefore, we conclude that both the Hfr and F<sup>-</sup> parent require a metabolizable energy source during conjugation, and we suggest that the Hfr and the F<sup>-</sup> require energy to initiate chromosome transfer and to control the rate of chromosome transfer, respectively.

TABLE 5. Recombinant production in matings with thymine-requiring parents<sup>a</sup>

Donor strain	Recipient strain	Thymidine present during mating	Recombinant frequency		Percentage of control		No. of comparative matings	Mean per cent inhibition
			<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>		
x 503 <i>purE</i> <sup>+</sup> <i>pyr</i> <sup>+</sup> <i>thy</i> <sup>+</sup> <i>str</i> <sup>a</sup>	x 724 <i>purE</i> <sup>-</sup> <i>pyr</i> <sup>-</sup> <i>thy</i> <sup>-</sup> <i>str</i> <sup>r</sup>	Yes	6.4 × 10 <sup>-1</sup>	8.1 × 10 <sup>-2</sup>	100	100	5	5
		No	6.1 × 10 <sup>-1</sup>	8.2 × 10 <sup>-2</sup>	95	101		
x 584 <i>thr</i> <sup>+</sup> <i>purE</i> <sup>+</sup> <i>thy</i> <sup>-</sup> <i>str</i> <sup>a</sup>	x 723 <i>thr</i> <sup>-</sup> <i>purE</i> <sup>-</sup> <i>thy</i> <sup>+</sup> <i>str</i> <sup>r</sup>	Yes	<i>thr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>thr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	6	42
		No	1.0 × 10 <sup>0</sup> 6.1 × 10 <sup>-1</sup>	4.5 × 10 <sup>-1</sup> 2.7 × 10 <sup>-1</sup>	100 61	100 60		
x 584 <i>thr</i> <sup>+</sup> <i>purE</i> <sup>+</sup> <i>thy</i> <sup>-</sup> <i>str</i> <sup>a</sup>	x 724 <i>thr</i> <sup>-</sup> <i>purE</i> <sup>-</sup> <i>thy</i> <sup>-</sup> <i>str</i> <sup>r</sup>	Yes	7.8 × 10 <sup>-1</sup>	1.6 × 10 <sup>-1</sup>	100	100	9	57
		No	3.7 × 10 <sup>-1</sup>	9.8 × 10 <sup>-2</sup>	47	61		

<sup>a</sup> The bacteria were grown to log phase in appropriately supplemented minimal medium containing 0.5% Casamino Acids and 0.5% glucose, the Hfr parent without and the F<sup>-</sup> parent with aeration. The cells were then sedimented, washed gently, and resuspended in prewarmed minimal mating medium, supplemented as above, but lacking thymidine. Cultures were then starved for 20 min with aeration to deplete the endogenous supply of thymine and its derivatives. Thymidine at a concentration of 4 μg/ml was added to the control 30 sec before commencement of mating. Matings were interrupted after 40 min by diluting into UV-irradiated T6 suspended in minimal medium supplemented to restore the concentration of thymidine to 4 μg/ml if thymine was required by the F<sup>-</sup> recipient. Unadsorbed T6 was neutralized with antiserum to T6. The donor and recipient cell titers in the mating mixture were determined at the beginning and end of the mating period. None of the *thy*<sup>-</sup> strains used exhibited thymineless death until after 60 min in the absence of thymine.

### Effects of Inhibiting DNA Synthesis Before and During Conjugation

**Recombinant production in matings with thymine-requiring parents.** Pritchard (48) used *thy*<sup>-</sup> Hfr and F<sup>-</sup> parents to show that starvation of both parents for thymine during mating did not alter the rate of chromosome transfer, but did decrease the total recombinant yield. He also obtained this effect on recombinant yield when only the Hfr *thy*<sup>-</sup> strain was deprived of thymine; Pritchard therefore suggested that DNA synthesis may be required in the Hfr to permit initiation of chromosome transfer. The maximal recombination frequencies in Pritchard's experiments were only about 5 to 10%. Thus, the average amount of chromosomal material transferred from each donor was low (see 53). It was therefore possible that the small amount of thymine synthesized by the possibly leaky, aminopterin-derived *thy*<sup>-</sup> Hfr mutant might be sufficient to allow replication of the Hfr chromosome during transfer according to the Jacob-Brenner-Cuzin (38) model.

We have repeated Pritchard's experiments using isogenic donor and recipient strains and under mating conditions in which essentially every Hfr cell was paired with one or more F<sup>-</sup> cells. Table 5 contains data from representative matings with aminopterin-derived *thy*<sup>-</sup> parents conducted in the presence and absence of thymidine. Summary values for the mean per cent inhibition in recombinant yield obtained for each type of mating performed in the absence of thymidine are also included. Thymine starvation has little effect on the *thy*<sup>-</sup> F<sup>-</sup> parent but has a significant effect on the *thy*<sup>-</sup> Hfr parent. The per cent inhibition when both *thy*<sup>-</sup> parents are deprived of thymine (57%) exceeds the combined inhibition when either *thy*<sup>-</sup> strain is singly deprived of thymine. This suggests that some supply of thymine or of its derivatives may be provided to the *thy*<sup>-</sup> parent by the *thy*<sup>+</sup> parent, either by cross-feeding through the medium or by cytoplasmic transfer, or by a combination of these methods. The per cent inhibition in recombinant frequency was usually greater for the proximally transferred marker than for the distally transferred marker (Table 5). We have no explanation for this observation.

Figure 2 presents results from interrupted matings conducted in the presence and absence of thymidine between thymine-requiring parents. The times of first appearance of the donor *thr*<sup>+</sup> and *purE*<sup>+</sup> markers in recombinants are the same in both matings. The times of attaining plateaus in recombinant frequencies appear to be slightly delayed in the mating conducted in the absence of thymidine, but the beginning of thymineless death of the F<sup>-</sup> parent, 70 min after removal of

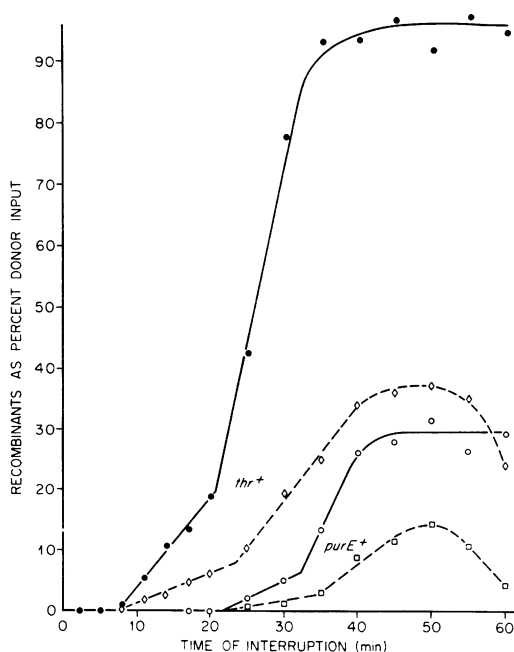


FIG. 2. Interrupted mating between thymine-requiring parents in the presence (solid lines) and absence (dashed lines) of 4  $\mu$ g of thymidine/ml.  $\chi$  584 (Hfr OR41 *thr*<sup>+</sup> *purE*<sup>+</sup> *thy*<sup>-</sup> *str*<sup>r</sup>) and  $\chi$  724 (F<sup>-</sup> *thr*<sup>-</sup> *purE*<sup>-</sup> *thy*<sup>-</sup> *str*<sup>r</sup>) were at titers of  $1.2 \times 10^7$ /ml and  $1.9 \times 10^8$ /ml, respectively in the mating mixtures. Other methods are described in the footnote to Table 5.

thymidine, complicates this type of analysis. Note that the frequency of recombination for the proximally transferred *thr*<sup>+</sup> marker approaches 100% in the mating with thymidine present (see also Table 5). If the probability of integrating a marker is 0.5 of the transfer frequency (39), then each Hfr cell in the control experiment presented in Fig. 2 must be transferring a partial chromosome to an average of two F<sup>-</sup> cells.

**DNA synthesis and initiation of chromosome transfer.** As a first guess as to why thymine starvation of *thy*<sup>-</sup> Hfr strains inhibited recombinant formation, we decided to test Pritchard's suggestion (48) that DNA synthesis was needed in the Hfr parent to initiate chromosome transfer. To do this, *thy*<sup>-</sup> parents were starved for thymine for 20 min, mated for 5 min in the presence of thymidine to allow specific pair formation and initiation of chromosome transfer, and then diluted 1:200 into medium with or without thymidine or thymine. The results (Table 6) indicate that 5 min in the presence of thymidine or thymine is sufficient to allow mating partners to give uninhibited recombinant frequencies during another 60 min of mating in the "absence" of thymine or thymidine. There are at least two types

of potential problems with this type of experiment. First, it is possible that the diluted thymine-requiring mating partners can obtain a sufficient amount of thymidine or thymine, which is present at 0.02  $\mu\text{g}/\text{ml}$ , from the medium to allow replication of the donor chromosomes during transfer. If uptake of thymidine or thymine were by simple diffusion, then there would be a sufficient amount within each cell to allow replication of about 10% of one chromosome per cell if the enzymes involved in this replication have high affinities for thymidine or thymine. If the thymidine or thymine is actively transported into the cells, then the concentration of thymidine or thymine in the medium is sufficient to allow extensive amounts of chromosome replication and cell growth. However, if such uptake occurs, the thymine or thymidine must be preferentially used for replication of the chromosome being transferred and not for vegetative chromosome replication, since thymineless death of both parents is occurring by 80 min after dilution of the mating mixture (85 min after commencement of mating). In control experiments, both parents commence thymineless death 70 min after removal of thymine from the growth medium (see Fig. 2). It thus seems unlikely that the thymine or thymidine at dilute concentration is being actively utilized by the *thy*<sup>-</sup> parents. The second difficulty concerns the fact that strains in the W1485 subline of *E. coli* K-12 possess a thymine pool sufficient to allow a mean replication of about 10% of one chromosome per cell (L. G. Caro, *personal communication*). Since the *purE*<sup>+</sup> marker is about 20 min of transfer time from the origin of chromosome transfer of Hfr OR41, it would again be necessary for the cells to use the thymine pool preferentially for the replication of the chromosome being transferred. Furthermore, in one experiment the *pyr*<sup>+</sup> marker, which is transferred 16 min after the *purE*<sup>+</sup> marker, was also used for recombinant selection, and the frequencies of *pyr*<sup>+</sup> recombinants after 60 min of mating were the same for the mating partners diluted into medium with and without thymidine.

The results presented in this section suggest, but do not prove, (i) that DNA synthesis is required in the Hfr during the beginning of mating to permit all the cells to initiate chromosome transfer and (ii) that although DNA synthesis may normally accompany chromosome transfer, as recent evidence seems to suggest (6, 33, 34, 49), it is not necessary for transfer and therefore does not control the rate of chromosome transfer. Our results with thymine-requiring strains are also in complete accord with those obtained by Pritchard (48).

#### *Effects of Inhibiting Protein Synthesis Before and During Conjugation*

*Effects of amino acid starvation on recombinant production.* Krisch and Kvetkas (41) and Fisher (29) both showed that amino acid starvation of amino acid-requiring Hfr strains reduced recombinant yield. They also showed that the inability of Suit et al. (54) to demonstrate an effect of amino acid starvation on Hfr parents was due to cross-feeding which occurred on the membrane filters used for matings. We have shown that starvation of donor strains for a required amino acid results in an exponential loss in number of F pili and in specific pair-forming ability, with a concomitant increase in recipient ability (i.e., phenocopy production). These results will be communicated in detail elsewhere (Curtiss and Caro, *in preparation*), and the data presented and discussed in this report principally concern other effects of amino acid starvation.

Table 7 presents data on the effects of amino acid starvation of either or both parents on recombinant production. The most striking feature of these data is the observation that amino acid starvation of the F<sup>-</sup> parent before and during mating results in an increase in recombinant frequency, especially for the more distally transferred *pyr*<sup>+</sup> marker. Amino acid starvation of the Hfr parent causes a decrease in recombinant yield with some, but not all, of the effect probably being due to loss of F pili (see below).

*Effects of completion of vegetative chromosome replication on recombinant production.* One well-known effect of amino acid starvation is that it allows completion of a round of vegetative chromosome replication without permitting initiation of a new round of replication (42, 46). The derivatives of the *E. coli* K-12 substrain W1485, specifically  $\chi$  584, behave in this manner (L. G. Caro, *personal communication*). We therefore performed experiments with both Hfr and F<sup>-</sup> strains to determine whether there was any correlation between recombinant-forming ability and any stage in the chromosome replication cycle.

Figure 3 presents data from an experiment in which  $\chi$  584, a *pro*<sup>-</sup> Hfr parent, was starved of proline to allow completion of vegetative chromosome replication. The results obtained from matings initiated during the 90-min starvation period for proline indicate that amino acid starvation causes a progressive loss in recombinant-forming ability. The addition of proline at the commencement of mating only gives a partial restoration of donor activity, which is not much greater for the proline-starved Hfr cultures than for the zero-time mating with the unstarved Hfr culture. This loss of donor ability which is restorable by addition

TABLE 6. *Effect of supplying thymidine or thymine to thymine-requiring parents during the early stages of mating<sup>a</sup>*

Expt	Presence of thymidine or thymine during		Recombinant per cent at						Cell titer in mating at						
	Starvation	Pair formation	Mating	20 min		40 min		60 min		5 min		55 min <sup>b</sup>		85 min <sup>b</sup>	
				<i>thr</i> <sup>+</sup>	<i>purE</i> <sup>+</sup>	<i>thr</i> <sup>+</sup>	<i>purE</i> <sup>+</sup>	<i>thr</i> <sup>+</sup>	<i>purE</i> <sup>+</sup>	Hfr	F <sup>-</sup>	Hfr	F <sup>-</sup>	Hfr	F <sup>-</sup>
1	Absent	Thymidine	Thymidine	21	1	64	22	79	34	1.1 × 10 <sup>7</sup>	2.0 × 10 <sup>8</sup>	8.7 × 10 <sup>8</sup>	2.2 × 10 <sup>8</sup>	1.0 × 10 <sup>7</sup>	2.5 × 10 <sup>8</sup>
	Absent	Thymidine	"Absent" <sup>e</sup>	19	0.5	67	23	75	35	1.1 × 10 <sup>7</sup>	1.9 × 10 <sup>8</sup>	8.5 × 10 <sup>8</sup>	2.0 × 10 <sup>8</sup>	1.6 × 10 <sup>8</sup>	4.3 × 10 <sup>7</sup>
2	Absent	Thymine	Thymine	11	0.6	39	13	52	24	1.6 × 10 <sup>7</sup>	1.3 × 10 <sup>8</sup>	1.9 × 10 <sup>7</sup>	1.2 × 10 <sup>8</sup>	2.1 × 10 <sup>7</sup>	1.7 × 10 <sup>8</sup>
	Absent	Thymine	"Absent" <sup>e</sup>	6.5	0.4	30	7	51	22	1.4 × 10 <sup>7</sup>	1.4 × 10 <sup>8</sup>	1.5 × 10 <sup>7</sup>	1.5 × 10 <sup>8</sup>	7.8 × 10 <sup>8</sup>	6.1 × 10 <sup>7</sup>

<sup>a</sup> The donor and recipient strains used were x 584 and x 724, respectively. After 20 min of starvation to remove endogenous thymine and its derivatives, the bacteria were allowed 5 min to form specific pairs in the presence of 4 μg/ml of thymidine (Expt 1) or thymine (Expt 2). The mating mixture was then gently diluted, with a pipette of constant bore, 1:200 into appropriately supplemented minimal medium containing Casamino Acids and glucose and with or without thymidine or thymine at 4 μg/ml. The titers of F<sup>-</sup> and Hfr cells were determined periodically by plating appropriate dilutions on media selective for each parentage. Other procedures are described in the footnote to Table 5.

<sup>b</sup> Actual titers 200 times less.

<sup>c</sup> Actual concentration of 0.02 μg/ml which is sufficient to allow replication of 10% of one chromosome per cell if there is no active transport of thymidine or thymine into the cell.

TABLE 7. *Effects of amino acid starvation on recombinant production<sup>a</sup>*

Parents	Amino acids omitted			Recombinant frequency	Percentage of control	
	30 min before mating		During mating			
	Hfr	F <sup>-</sup>				
χ 503 (Hfr OR21 <i>thr</i> <sup>+</sup> <i>purE</i> <sup>+</sup> <i>pyr</i> <sup>+</sup> <i>his</i> <sup>+</sup> <i>str</i> <sup>+</sup> ) × χ 820 (F <sup>-</sup> <i>thr</i> <sup>-</sup> <i>purE</i> <sup>-</sup> <i>pyr</i> <sup>-</sup> <i>his</i> <sup>-</sup> <i>str</i> <sup>r</sup> )	None None None	None Thr, His Thr, His	None None Thr, His	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	100 118 111  16 (100) 34 (211) 36 (225)
χ 584 (Hfr OR41 <i>thr</i> <sup>+</sup> <i>proB</i> <sup>-</sup> <i>purE</i> <sup>+</sup> <i>str</i> <sup>+</sup> ) × χ 723 (F <sup>-</sup> <i>thr</i> <sup>-</sup> <i>pro</i> <sup>+</sup> <i>purE</i> <sup>-</sup> <i>str</i> <sup>r</sup> )	None Pro Pro	None None None	None None Pro	<i>thr</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>thr</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup>	100 76 60  41 (100) 33 (79) 24 (58)
χ 584 (Hfr OR41 <i>thr</i> <sup>+</sup> <i>proB</i> <sup>-</sup> <i>purE</i> <sup>+</sup> <i>str</i> <sup>+</sup> ) <sup>b</sup> × χ 821 (F <sup>-</sup> <i>thr</i> <sup>-</sup> <i>proA</i> <sup>-</sup> <i>purE</i> <sup>-</sup> <i>str</i> <sup>r</sup> ) <sup>b</sup>	None Pro Pro	None Pro Pro	None None Pro	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	<i>purE</i> <sup>+</sup> <i>str</i> <sup>r</sup> <i>pyr</i> <sup>+</sup> <i>str</i> <sup>r</sup>	100 80 70  16 (100) 23 (141) 20 (121)

<sup>a</sup> Bacteria were grown to log phase in appropriately supplemented minimal medium which contained all 20 amino acids and glucose; the recipient strains were grown with and the donor strains without aeration. This medium was nearly equal to one supplemented with Casamino Acids with respect to F pilation and pair-forming ability of donor strains. All cultures were sedimented, gently washed, and resuspended in prewarmed minimal medium lacking one or two amino acids as indicated. These amino acids were added back immediately to the cultures to be mated with no starvation prior to or during mating and were added 30 sec before mating to the cultures starved for 20 min prior to mating in the presence of the amino acids. The matings were interrupted with UV-irradiated T6 after 40 min.

<sup>b</sup> Strains having *proA*<sup>-</sup> mutations are incapable of crossfeeding strains with *proB*<sup>-</sup> mutations and vice versa (18).

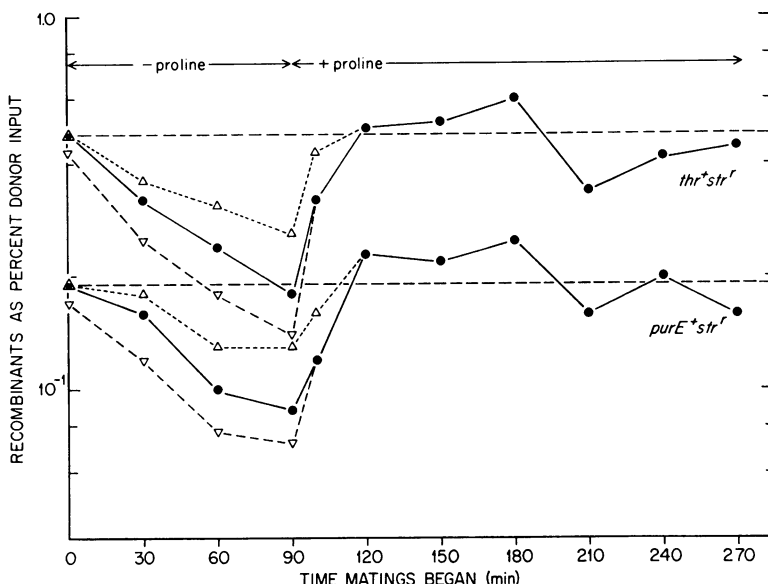


FIG. 3. Effects of amino acid starvation and completion of vegetative chromosome replication on recombinant yield by an Hfr parent.  $\chi$  584 (Hfr OR41  $thr^+ pro^- purE^+ str^+$ ) and  $\chi$  723 ( $F^- thr^- pro^+ purE^- str^+$ ) were used. Media and procedures for growth, starvation, and mating were as described in the footnote to Table 7. The experiment was initiated immediately after resuspension of the washed  $\chi$  584 culture in medium devoid of proline. A separate aerated culture of  $\chi$  723 was used for each mating. Upon addition of proline to the  $\chi$  584 culture, periodic dilutions with prewarmed medium were made to maintain the density at about  $10^8$  cells/ml. The matings were interrupted after 33 min. Recombinant frequency values obtained in matings performed in the presence of proline are connected by solid lines and those obtained in the absence of proline by dashed lines. The points connected by dotted lines are the values for matings in the presence of proline corrected for loss of F pili.

of proline to the mating mixture might be due to the inability of amino acid-starved donors to initiate chromosome transfer, as was suggested by Fisher (29). The nonrestorable loss of donor ability during amino acid starvation is therefore probably due to loss of structural components necessary for conjugation which cannot be sufficiently resynthesized within the mating period to restore normal donor activity. F pili, which are necessary for specific pair formation, are lost at an exponential rate during amino acid starvation (Curtiss and Caro, *Bacteriol. Proc.*, p. 27, 1966; *manuscript in preparation*), and we have thus corrected the recombinant frequencies to account for the decrease in mean number of F pili per cell (Fig. 3). Even though no correction was applied to the data for any decrease in F pili length, it seems reasonable to assume that some other component necessary for conjugation is also lost during amino acid starvation. Based on studies with the minicell-producing mutant isolated and described by Adler et al. (3), Cohen et al. (14, 16) inferred that donor and recipient cells differ with respect to a cell surface component in addition to the presence or absence of F pili. Although it was suggested that this cell surface

component was involved in pair formation, the experiments did not permit the distinction as to whether the cell surface component(s) was on the donor or the recipient cell, or on both. We (Curtiss and Stallions, *unpublished data*) have recently isolated two classes of mutant donor strains which have relevance to this discussion, one which has F pili but is unable to donate genetic material and the other which lacks F pili but has the recipient ability characteristic of the parental donor strain. Mutants of the first type can form specific pairs with  $F^-$  cells, whereas mutants of the second type cannot. Mutants of the second type form specific pairs with donor cells infrequently. On the basis of all of these observations, we suggest that much of the loss of donor ability during amino acid starvation, which is not restored by addition of proline to the mating and which is not accounted for by F pili loss, is probably due to loss of a donor cell surface component necessary for effective pair formation.

If ability of Hfr donor cells to initiate chromosome transfer were correlated with some stage in the vegetative chromosome replication cycle, as is called for in the Bouck-Adelberg (8) model for

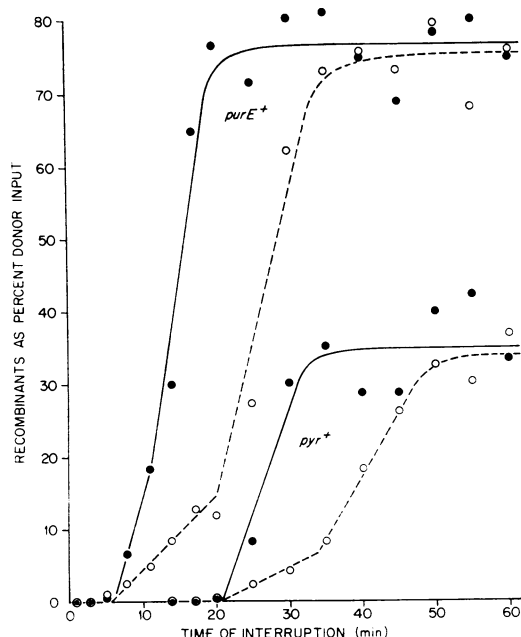


FIG. 4. Effects of amino acid starvation and completion of vegetative chromosome replication on recombinant yield by an  $F^-$  parent.  $\chi$  503 ( $Hfr$  OR21  $thr^+$   $purE^+$   $pyr^+$   $his^+$   $str^s$ ) and  $\chi$  820 ( $F^-$   $thr^-$   $purE^-$   $pyr^-$   $his^-$   $str^s$ ) were used. Media and procedures for growth, starvation, and mating were as described in the footnote to Table 7.  $\chi$  820 was starved (solid lines) or not starved (dashed lines) for histidine and threonine for 60 min prior to mating. Histidine and threonine were added to the starved  $\chi$  820 culture 30 sec before addition of the  $Hfr$  parent.

chromosome transfer, then it might be expected that recombinant frequencies would fluctuate in some cyclic manner after readdition of proline to the  $Hfr$  culture. This type of result was not observed, and the recombinant frequencies obtained from matings initiated after proline was restored to the culture do not vary significantly from those obtained in the mating of the untreated controls (Fig. 3). There was also no observed change in the kinetics of recombinant formation associated with completion of vegetative chromosome replication as measured in interrupted mating experiments (data not shown). Synchronous reinitiation of DNA synthesis upon addition of proline to the  $Hfr$  culture at 90 min may be poor. However, the same type of experiment as presented in Fig. 3 has been done with the  $F^+$  strain from which  $\chi$  584 was isolated, and the results indicate a correlation between donor ability and some stage in the vegetative chromosome replication cycle (23). The results presented in Fig. 3 do not, therefore, favor any model which

requires a correlation between a specific stage in the vegetative chromosome replication cycle and ability to initiate chromosome transfer by  $Hfr$  donors.

Figure 4 presents results of an interrupted mating experiment with an amino acid-requiring  $F^-$  strain which either has been starved of amino acids for 60 min or has not been starved. Amino acid starvation has dramatically altered the apparent kinetics of chromosome transfer. The length of mating time between the time of first appearance of each  $Hfr$  marker in recombinants and the time a plateau in the frequency of that recombinant type is attained has been cut in half in the mating with the amino acid-starved  $F^-$ . The times of first appearance of each  $Hfr$  marker in recombinants are unchanged by using an amino acid-starved  $F^-$ , and we therefore conclude that there is no change in the rate of chromosome transfer. The experiment presented in Fig. 4 provides an explanation for the data in Table 7 which showed that amino acid starvation of an amino acid-requiring  $F^-$  caused increased recombinant yields, especially for  $Hfr$  markers located distally from the origin of chromosome transfer. The matings used to collect the data for Table 7 were interrupted after 40 min. It can be seen in Fig. 4 that the frequency of  $pyr^+$  recombinants is still increasing at 40 min in the mating with the unstarved  $F^-$ , while it has already reached a plateau in the mating with the amino acid-starved recipient.

There are at least two possible explanations for the results obtained with amino acid-starved  $F^-$  recipients. In the first, it is suggested that vegetative chromosome replication must reach a specific stage (e.g., completion) before the  $F^-$  can actively participate in the transfer of the donor chromosome with an expenditure of energy. In the second, it is suggested that amino acid starvation alters the physiology of the recipient so that markers which are nearest the end of the chromosome broken at the time of interruption are not lost by nuclease degradation. A corollary of this second suggestion is that, under normal mating conditions, the probability of integrating a marker transferred into the recipient just before the time of interruption is much less than 0.5. This second possible explanation was kindly provided by Charles Brinton (*personal communication*). The use of zygotic induction did not allow a distinction between these two hypotheses, but other experiments are being conducted to prove or disprove the first explanation.

In summary, amino acid starvation of  $Hfr$  donors causes (i) a loss of  $F$  pili and ability to form specific pairs with  $F^-$  cells (Curtiss and

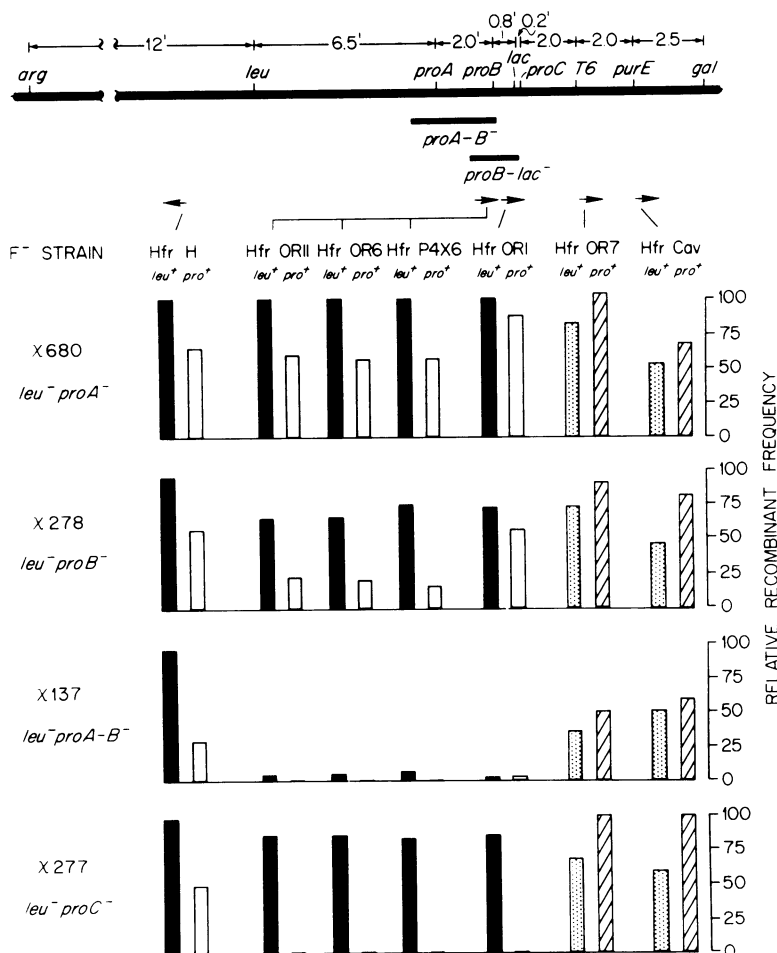


FIG. 5. The *pro-lac* region of the *E. coli* chromosome, the origins and directions of chromosome transfer by Hfr donors, and the effect of a *proA-B<sup>-</sup>* deletion mutation on recombinant yield. The *F<sup>-</sup>* strains  $\chi$  680 (*proA<sup>-</sup>*),  $\chi$  278 (*proB<sup>-</sup>*),  $\chi$  277 (*proC<sup>-</sup>*), and  $\chi$  137 (*proA-B<sup>-</sup>*) are all closely related and are descended from the C600 subline. Hfr H ( $\chi$  57), Hfr P4  $\times$  6 ( $\chi$  313) and Hfr Cav ( $\chi$  235) are all descended from the 58-161 subline. Hfr ORI ( $\chi$  225) and Hfr OR11 ( $\chi$  493) were isolated from the same *F<sup>+</sup>* strain and are descended from W1485. Hfr OR6 ( $\chi$  435) and Hfr OR7 ( $\chi$  436) are closely related and are descended from the K-12-112 subline. The data presented were obtained by mating each Hfr strain with the four *F<sup>-</sup>* strains on two to five different occasions. The average recombinant frequencies were used to calculate relative values. (The frequency of *leu<sup>+</sup>* recombinants in crosses with  $\chi$  680 or of *proC<sup>+</sup>* recombinants in crosses with  $\chi$  277 was equated to 100.) Bacteria were grown and mated in L broth. Matings were of 40-min duration.

Caro, Bacteriol. Proc., p. 27, 1966; manuscript in preparation), (ii) a possible loss of a structural component of the donor cell surface which is needed to establish effective pairs with *F<sup>-</sup>* cells and prevents donor-donor matings, and (iii) a possible inability to synthesize a specific protein needed by the Hfr to initiate chromosome transfer (29). Amino acid starvation of the *F<sup>-</sup>* recipient alters the apparent kinetics of chromosome transfer by either of two presently indistinguishable mechanisms.

#### Effects of Genetic Inhomology Between Donor and Recipient Chromosomes on Chromosome Transfer

In 1964, Johnson, Falkow, and Baron (40) demonstrated in matings between Hfr strains of *E. coli* K-12 and recipient strains of *Salmonella typhosa* that large increases in recombinant frequency were obtained when the recipient was a hybrid containing *E. coli* genetic material. However, these increased recombinant frequencies were only observed when the hybrid recipient

contained genetic material from the proximally transferred portion of the Hfr chromosome, but not when such hybrids contained distally transferred *E. coli* genetic material. We (20) have observed a similar requirement for genetic homology between the lead region of the Hfr chromosome and the comparable portion of the F<sup>-</sup> chromosome in *E. coli* K-12.

*Effects of deletions in the F<sup>-</sup> chromosome on recombinant production by Hfr donors.* The diagram at the top of Fig. 5 provides a map of the portion of the *E. coli* chromosome used for these studies, with the origins and directions of chromosome transfer for seven Hfr strains. Our initial observation of an effect of genetic inhomology on recombinant yield was obtained in matings in which F<sup>-</sup> strains possessed the *proA-B* deletion mutation. This mutation deletes a segment of chromosome which includes the *proA* and *proB* cistrons and, in addition to causing a requirement for proline, confers resistance to four phages (18). Several independently isolated *proA-B*<sup>-</sup> mutations have been intensively studied, and results from a variety of genetic tests have indicated that all have the same termination point between the *proB* and *lac* loci (Curtiss and Charamella, unpublished data). However, the termination point between the *argF* and *proA* loci (see 55) has not been determined, and therefore the segment deleted in *proA-B*<sup>-</sup> strains is between 2.2 and 4.5% of the chromosome.

The data presented in Fig. 5 summarize the results of matings between seven Hfr strains and F<sup>-</sup> strains having either a point mutation at the *proA*, *proB*, or *proC* locus or a *proA-B* deletion mutation. All four F<sup>-</sup> strains had the same *leu*<sup>-</sup> mutation. In the matings with the *proA*<sup>-</sup> F<sup>-</sup> ( $\chi$  680), all Hfr donors gave results which were similar and reasonably normal. The only discrepancy from expectation was the fact that the frequency of *proA*<sup>+</sup> recombinants did not exceed the frequency of *leu*<sup>+</sup> recombinants in the matings with Hfr OR11, Hfr OR6, Hfr P4X6, and Hfr OR1. This is a property of all *proA*<sup>-</sup> derivatives of the C600 subline and is not found with *proA*<sup>-</sup> mutants of the W945 F<sup>-</sup> subline. In the matings with the *proB*<sup>-</sup> F<sup>-</sup> ( $\chi$  278), there is a significant reduction in the frequency of *proB*<sup>+</sup> recombinants in matings with Hfr OR11, Hfr OR6, and Hfr P4X6. This is probably due to the proximity of the *proB* locus to the origins of chromosome transfer for these Hfr strains (32, 45, 47, 58). The frequencies of *leu*<sup>+</sup> recombinants are, however, very similar to the frequencies of *leu*<sup>+</sup> recombinants found in matings with  $\chi$  680 and  $\chi$  277. In the mating in which the F<sup>-</sup> has the *proA-B*<sup>-</sup> deletion mutation ( $\chi$  137), some of the results differ significantly from those obtained in

matings with F<sup>-</sup> strains having point mutations. The frequency of *leu*<sup>+</sup> recombinants is normal in matings with Hfr H and Hfr Cav, and is reduced by a factor of two in the mating with Hfr OR7. However, the frequency of *leu*<sup>+</sup> recombinants is reduced 24-, 18-, 14-, and 34-fold in matings with Hfr OR11, Hfr OR6, Hfr P4X6, and Hfr OR1, respectively. Thus, a significant reduction in the frequency of *leu*<sup>+</sup> recombinants is only achieved when there is a large deletion in the F<sup>-</sup> chromosome for the segment of chromosome first transferred by an Hfr donor.

Similar results leading to the same conclusion were obtained from matings between the Hfr donors used to obtain the data in Fig. 5 and a series of proline-deficient F<sup>-</sup> strains derived from the W945 subline of *E. coli* K-12 (data not shown).

To obtain more information about the requirement for genetic homology between the lead region of the Hfr chromosome and the comparable portion of the F<sup>-</sup> chromosome, we initiated studies with isogenic strains derived from the W1485 subline. Hfr OR1, which has F integrated into the right end (Fig. 5) of the structural gene for  $\beta$ -galactosidase (*lacZ*; Curtiss, Bacteriol. Proc., p. 30, 1964), was used as the donor. A series of F<sup>-</sup> strains was prepared having the same *arg*<sup>-</sup> and *leu*<sup>-</sup> alleles but with different *proA*<sup>-</sup>, *proB*<sup>-</sup>, and *lac*<sup>-</sup> point mutations and with *proA-B*<sup>-</sup>, *proB-lac*<sup>-</sup>, and *lacZ-I*<sup>-</sup> deletion mutations (see Table 1). The results from representative interrupted matings between Hfr OR1 and these F<sup>-</sup> strains are presented in Fig. 6. Recombinant frequencies for each selected marker are lowest when the F<sup>-</sup> possesses a *proA-B*<sup>-</sup> deletion mutation, are significantly reduced when the F<sup>-</sup> possesses a *proB-lac*<sup>-</sup> deletion mutation, and are essentially unaffected when the F<sup>-</sup> has the *lacZ-I*<sup>-</sup> deletion mutation.

When the data from matings with F<sup>-</sup> strains having deletions are plotted with nearly the same ordinate scale used for plotting data from the control matings, there is an apparent long delay in the time of first appearance of each donor marker in recombinants with respect to marker entry times in the control matings (see Fig. 6A and C). However, when these data are replotted with the use of an expanded ordinate scale (Fig. 6B), it can be seen that the delay in marker entry time is much less, although still highly significant. (Note that all of these matings were done with a donor-to-recipient cell ratio of between 1:2 and 1:3 to facilitate detection of early formation of rare recombinants.)

The data presented in Fig. 6 and obtained from other matings with the W1485-derived F<sup>-</sup> strains are summarized in Table 8. First, by comparing

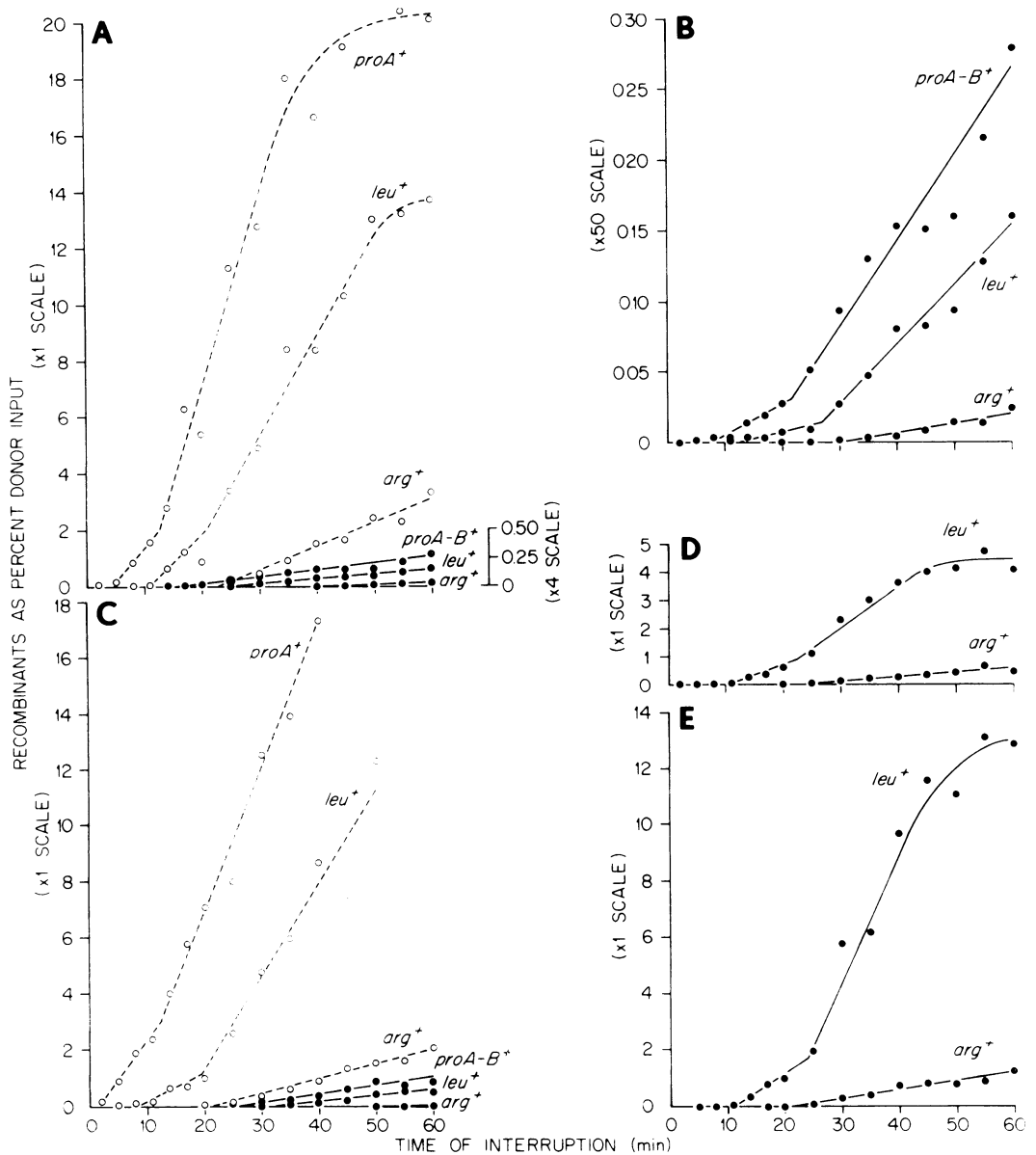


FIG. 6. Results of interrupted matings between Hfr ORI and F<sup>-</sup> strains having point or deletion mutations in the region of chromosome first transferred by Hfr ORI. (A) Hfr ORI ( $\chi$  225) ×  $\chi$  710 (*proA<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup>*) (dashed lines and left-hand ordinate scale) and Hfr ORI ×  $\chi$  711 (*proA-B<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup>*) (solid lines and right hand × 4 expanded scale). (B) Hfr ORI ×  $\chi$  711 data shown in Fig. 6A using a × 50 expanded ordinate scale. (C) Another set of matings between Hfr ORI and  $\chi$  710 (dashed lines) and Hfr ORI and  $\chi$  711 (solid lines). (D) Hfr ORI ×  $\chi$  784 (*proB-lac<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup>*). (E) Hfr ORI ×  $\chi$  733 (*lac Z-I<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup>*). Bacteria were grown and mated in L broth. The donor-to-recipient cell ratios were between 1:2 and 1:3 in the mating mixtures.

the mean recombinant frequencies for matings with  $\chi$  710 with those from matings with  $\chi$  711, it is observed that the *pro<sup>+</sup>*, *leu<sup>+</sup>*, and *arg<sup>+</sup>* recombinant frequencies are depressed 82, 95, and

255 times, respectively, when the F<sup>-</sup> possesses the *proA-B<sup>-</sup>* deletion. The normal marker entry times for *pro<sup>+</sup>*, *leu<sup>+</sup>*, and *arg<sup>+</sup>* are also delayed 7.3, 6.0, and 8.0 min. respectively, if the F<sup>-</sup> recipient has

TABLE 8. Recombinant frequencies and marker entry times obtained in matings with *F<sup>-</sup>* strains having point or deletion mutations in the region of chromosome first transferred by *Hfr* OR1<sup>a</sup>

<i>F<sup>-</sup></i> strain and genotype	Recombinant frequency (%) after 40 min of mating			Marker entry times (min)					
	<i>pro<sup>+</sup></i>	<i>leu<sup>+</sup></i>	<i>arg<sup>+</sup></i>	<i>pro<sup>+</sup></i>		<i>leu<sup>+</sup></i>		<i>arg<sup>+</sup></i>	
				× 1 scale <sup>b</sup>	Expanded scale <sup>b</sup>	× 1 scale	Expanded scale	× 1 scale	Expanded scale
<i>x</i> 710 <i>proA<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup> str<sup>r</sup></i>									
Expt 1 (Fig. 6A)	16.6	8.4	1.5	4.2	—	10.7	—	22.8	—
Expt 2 (Fig. 6C)	17.3	8.7	1.0	1.5	—	8.0	—	21.0	—
Expt 3	17.6	8.6	1.4	3.9	—	10.3	—	21.4	—
Mean	17.2	8.6	1.3	3.2	—	9.7	—	21.7	—
Relative Mean	100	50	7.6						
<i>x</i> 711 <i>proA<sup>-</sup>B<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup> str<sup>r</sup></i>									
Expt 1 (Fig. 6A, B)	0.15	0.080	0.0042	16	7.8 (× 50)	23	12.5 (× 50)	40	30 (× 50)
Expt 2 (Fig. 6C)	0.38	0.14	0.0074	25.5	12.2 (× 20)	31.5	17.5 (× 20)	50	30 (× 20)
Expt 3	0.11	0.053	0.0038	22	11.5 (× 100)	30	17.1 (× 100)	45	29 (× 100)
Mean	0.21	0.091	0.0051	21.2	10.5	28.2	15.7	45	29.7
Relative mean	100	43	2.4						
<i>x</i> 784 <i>proB<sup>-</sup>lac<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup> str<sup>r</sup></i>									
Expt 1 (Fig. 6D)	—	3.6	0.25	—	—	10.5	10.3 (× 4)	22.5	22.0 (× 4)
Expt 2	—	1.4	0.21	—	—	14.5	13.6 (× 10)	29	26.0 (× 10)
Mean	—	2.5	0.23	—	—	12.5	12.0	25.8	24.0
Relative mean	—	100	9.2						
<i>x</i> 733 <i>lacZ<sup>-</sup>F<sup>-</sup> leu<sup>-</sup> arg<sup>-</sup> str<sup>r</sup></i>									
Expt 1 (Fig. 6E)	—	9.7	0.76	—	—	11.2	—	22.0	—
Expt 2	—	6.6	0.39	—	—	9.6	—	21.5	—
Mean	—	8.2	0.58	—	—	10.4	—	21.8	—
Relative mean	—	100	7.1						

<sup>a</sup> All data were obtained from matings performed as described in the legend to Fig. 6.

<sup>b</sup> Data from matings in which the *F<sup>-</sup>* possessed a deletion mutation for a region of chromosome first transferred by *Hfr* OR1 were plotted on graphs with an expanded ordinate scale in order to determine marker entry times accurately.

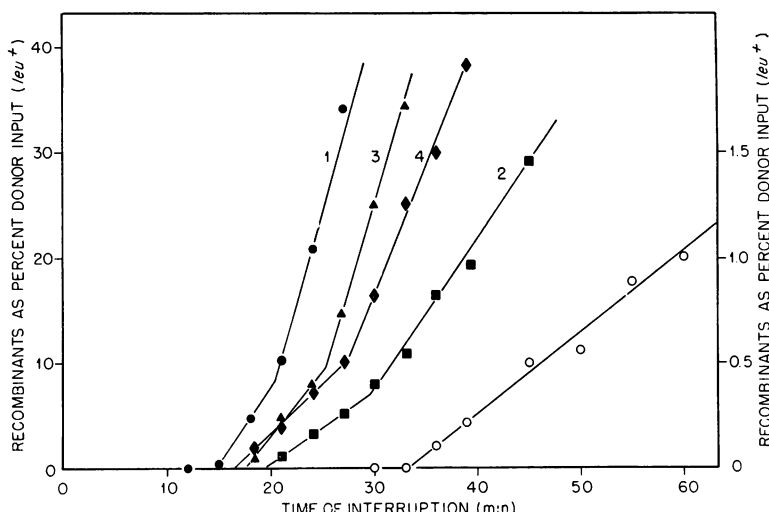


FIG. 7. Interrupted matings to select  $leu^+ str^+$  recombinants using four Hfr OR1 strains having independently isolated  $proA-B$  deletion mutations (closed symbols and left ordinate) and the Hfr OR1 parent ( $\chi$  225; open circles and right ordinate).  $\chi$  711 ( $F^- proA-B^- leu^- str^+$ ) was used as the recipient and was at a titer of about  $2 \times 10^8$ /ml in the mating mixtures. The Hfr titers were  $10^7$  to  $2 \times 10^7$ /ml in the mating mixtures.

the  $proA-B^-$  deletion. The data from matings with  $\chi$  784, which has a  $proB-lac^-$  deletion, indicate that  $leu^+$  and  $arg^+$  recombinant frequencies are reduced 3.4 and 5.7 times, respectively, and that  $leu^+$  and  $arg^+$  entry times are each delayed by 2.3 min. The  $lacZ-I^-$  deletion in  $\chi$  733 exerts no significant effect either on recombinant frequencies or on marker entry times (Table 8).

**Effects of deletions in the Hfr chromosome on recombinant production.** To eliminate any spurious explanations for the effects noted above, it is necessary to show that "normal" behavior is restored in matings between Hfr and  $F^-$  strains having the same deletion mutation. Unfortunately, the  $proB-lac^-$  deletion spans the integrated F in Hfr OR1 and the  $proA-B^-$  deletion mutation confers resistance to the transducing phage P1kc. Therefore, it was not possible to introduce any of the identical deletion mutations from the  $F^-$  strains into Hfr OR1. However, the  $proA-B^-$  deletions are probably ditto deletions with similar, if not identical, ends, as has already been mentioned. Therefore, several independent  $proA-B^-$  deletion mutants of Hfr OR1 were selected by challenge with phage T7 and replica plating to proline-deficient medium (18). When these Hfr OR1  $proA-B^-$  donors are mated with  $\chi$  710, an  $F^-$  with a  $proA^-$  point mutation, normal recombinant frequencies and entry times are obtained for  $leu^+$  and  $arg^+$  (data not shown). Figure 7 presents data from matings between four of these Hfr OR1  $proA-B^-$  mutants and the  $proA-B^- F^-$

$\chi$  711, and also data from a mating between the original Hfr OR1 and  $\chi$  711. The introduction of the  $proA-B^-$  deletion into the Hfr restored "normal" frequencies of inheritance and entry times for the  $leu^+$  marker.

All of the results presented in this and the preceding section indicate that genetic homology between the lead region of the Hfr chromosome and the comparable section of the  $F^-$  chromosome is required for early appearance of markers in recombinants and maximal frequencies of recombinant formation. There are two possible interpretations of these results. First, a lack of genetic homology between the lead region of the Hfr chromosome and the comparable segment of the  $F^-$  chromosome could have no effect on chromosome transfer but prevent the association between a normally transferred donor chromosome and the recipient chromosome, with a resultant loss of recombinants. Pittard and Walker (47) have obtained data which indicate that an interaction between a region near the leading end of the donor chromosome and the comparable segment of the recipient chromosome is associated with recombinant production. Second, it is possible that effective homologous pairing between the lead region of the Hfr chromosome and the comparable segment of the  $F^-$  chromosome must be established before an appreciable amount of chromosome transfer can occur. These two hypotheses are not mutually exclusive and together may account for the results obtained. A means of measuring chromo-

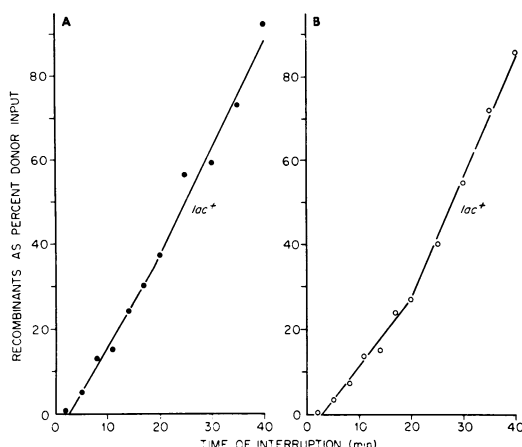


FIG. 8. *F'* *lac*<sup>+</sup> transfer to *F*<sup>-</sup> strains with a *lac*<sup>-</sup> point mutation ( $\chi$  708, A) or a *proB-lac* deletion mutation ( $\chi$  314, B).  $\chi$  314 was used as the *F'* donor and *lac*<sup>+</sup> *str*<sup>r</sup> recombinants were selected. Bacteria were grown and mated in L broth. The donor to recipient cell ratio was about 1:20. All (100 of 100) of the *lac*<sup>+</sup> *str*<sup>r</sup> recombinants in both matings were partially diploid *F' lac*<sup>+</sup> *lac*<sup>-</sup> donors.

some transfer which is independent of recombinant production must be used to distinguish between these two models.

**Effects of deletions in the *F*<sup>-</sup> chromosome on *F'* transfer.** The occurrence of haploid *F'* strains (5, 51) indicates that the replication of and expression of markers on *F'* episomes does not require the presence of the segment of chromosome homologous to the *F'* episome. Thus, expression of *F'* markers after conjugal transfer should be unaffected by a deletion in the recipient chromosome for part or all of the segment homologous to the genetic content of the *F'*. Therefore, studies on *F'* transfer to recipients with and without deletions corresponding to regions of the *F'* episome afford a means to determine whether homologous pairing between the lead region of the donor chromosome and the comparable segment of the recipient chromosome is needed for integration or for transfer of donor genetic material.

The kinetics and frequencies of transfer of the *F' lac*<sup>+</sup> episome, isolated by Jacob and Adelberg (37), to *F*<sup>-</sup> recipients having either a *lac*<sup>-</sup> point mutation or a *proB-lac*<sup>-</sup> deletion mutation are shown in Fig. 8. (All of the chromosomal material present in the *F' lac*<sup>+</sup> episome is missing from the chromosome of *F*<sup>-</sup> strains with the *proB-lac*<sup>-</sup> deletion.) As is shown in Fig. 8, the frequencies and rates of transfer of the *F' lac*<sup>+</sup> episome to both recipients are indistinguishable. Thus, homologous pairing between the *lac* regions of the

*F'* and chromosome is not necessary for the efficient transfer of the *F' lac*<sup>+</sup> episome. The transfer of the *F* episome also must not depend on homologous pairing between *F* and the chromosome. The observed low probability of *F* integration per bacterium per generation (ca.  $3 \times 10^{-6}$ ; Curtiss and Stallions, *Bacteriol. Proc.*, p. 55, 1968) suggests that only a limited homology exists between *F* and the chromosome, and yet *F* is rapidly transferred to *F*<sup>-</sup> recipients during conjugation with *F*<sup>+</sup> donors.

The kinetics and frequencies of transfer of the *F' lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> episome ORF-206 (5) to *F*<sup>-</sup> recipients having either a *proA*<sup>-</sup> point mutation or a *proA-B*<sup>-</sup> deletion mutation are shown in Fig. 9. The *proA-B*<sup>-</sup> mutation deletes the segment of chromosome first transferred by *F'* ORF-206 and causes about a 100-fold reduction in *F'* transfer. Thus, it appears that effective homologous pairing is required for the transfer of the *F'* ORF-206 episome. As was stated in the discussion of results obtained from Hfr matings with *F*<sup>-</sup> strains with the *proA-B*<sup>-</sup> deletion mutation, the *proA-B*<sup>-</sup> mutation does not appreciably affect recombinant yield in matings with Hfr donors which possess origins of chromosome transfer distal from the *lac* to *proA* segment. To eliminate the remote possibility that the *proA-B*<sup>-</sup> deletion mutation causes some type of restriction and loss of *F'* episomes, matings were performed between a donor with the KLF-1 episome (*thr*<sup>+</sup> *leu*<sup>+</sup> *F*) isolated by B. Low and the *F*<sup>-</sup> strains used in these studies. The data presented in Table 9 show that the frequencies of *leu*<sup>+</sup> recombinants are essentially the same for *F*<sup>-</sup> strains having *proA*<sup>-</sup> and *lac*<sup>-</sup> point mutations and *proB-lac*<sup>-</sup> and *proA-B*<sup>-</sup> deletion mutations.

Table 10 presents additional data on *F'* and chromosome transfer by *F' lac*<sup>+</sup> and *F' lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> donors to the same four *F*<sup>-</sup> strains used to obtain the results presented in Table 9. *F' lac*<sup>+</sup> transfer is unaffected by deletion of the *proB-lac* segment (mating 3 versus mating 1, Table 10), whereas the frequency of recombinants which arise as a consequence of chromosome mobilization and transfer by the *F' lac*<sup>+</sup> episome is slightly depressed when the *F*<sup>-</sup> has the *proB-lac*<sup>-</sup> deletion and is greatly depressed when the *F*<sup>-</sup> has the *proA-B*<sup>-</sup> deletion (matings 3 and 4, respectively, Table 10). In fact, the depressions in the frequencies of *pro*<sup>+</sup>, *leu*<sup>+</sup>, and *arg*<sup>+</sup> recombinants in matings with the *F*<sup>-</sup> strains  $\chi$  784 and  $\chi$  711 are very similar to those observed in the matings with Hfr OR1 (Fig. 6, Table 8). Transfer of the *F' lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> factor is reduced almost 100-fold in the mating with the *F*<sup>-</sup> having the *proA-B* deletion (mating 8, Table 10), but is unaffected when the *F*<sup>-</sup> possesses the *proB-lac*

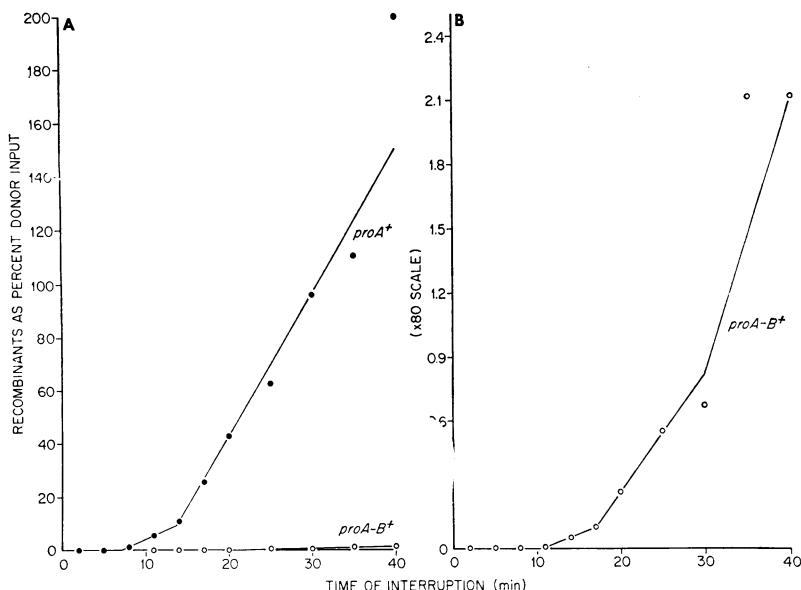


FIG. 9.  $F'$   $lac^+$   $proB^+$   $proA^+$  transfer to  $F^-$  strains with either a  $proA^-$  point mutation ( $\chi$  710) or a  $proA-B^-$  deletion mutation ( $\chi$  711). (A)  $\chi$  646 ( $F'$  ORF-206)  $\times$   $\chi$  710 with selection of  $proA^+$   $str^r$  recombinants ( $\bullet$ ) and  $\chi$  646  $\times$   $\chi$  711 with selection of  $proA-B^+$   $str^r$  recombinants ( $\circ$ ). (B). Data from the  $\chi$  646  $\times$   $\chi$  711 mating plotted with a  $\times 80$  ordinate scale. The bacteria were grown and mated in L broth. The donor-to-recipient cell ratio was about 1:10. Five per cent of the  $proA^+$  recombinants in the  $\chi$  646  $\times$   $\chi$  710 mating were haploid and nondonors. All other  $pro^+$  recombinants in both matings were partially diploid donors of  $F'$  ORF-206.

TABLE 9.  $F'$   $leu^+$  transfer to  $leu^-$   $F^-$  strains with either deletion or point mutations in the  $lac$  to  $proA$  region of their chromosomes<sup>a</sup>

Mating	Inheritance of $leu^+$ allele %
$\chi$ 927 $F'$ $thr^+$ $leu^+$ / $thr^-$ $leu^-$ $\times$	
(1) $\chi$ 708 $F^-$ $lacZ^-$ $proB^-$ $leu^-$ $arg^-$ . . .	73
(2) $\chi$ 710 $F^-$ $proA^-$ $leu^-$ $arg^-$ . . . . .	56
(3) $\chi$ 784 $F^-$ $proB-lac^-$ $leu^-$ $arg^-$ . . . . .	115
(4) $\chi$ 711 $F^-$ $proA-B^-$ $leu^-$ $arg^-$ . . . . .	43

<sup>a</sup> The bacteria were grown and mated in L broth. The matings were interrupted after 40 min by diluting the mating mixtures into minimal medium containing glucose, streptomycin, and 10% L broth (v/v) and immediately agitating the diluted mixtures for 60 sec on a vortex mixer. Further dilutions and plating were done after another 15 min at 37 C. The titers of  $\chi$  927,  $\chi$  708,  $\chi$  710,  $\chi$  784, and  $\chi$  711 in the mating mixtures were  $8.7 \times 10^6$ ,  $1.4 \times 10^8$ ,  $1.3 \times 10^8$ ,  $2.8 \times 10^8$ , and  $8.4 \times 10^7$ /ml, respectively.

deletion (mating 7, Table 10). The  $proA-B$  deletion mutation deletes the segment of chromosome comparable to the leading end of the  $F'$   $lac^+$   $proB^+$   $proA^+$  episome, whereas the  $proB-lac$  deletion deletes the segment comparable to the distally

transferred end of the episome. Thus, effective homologous pairing between the proximally transferred end of the  $F'$   $lac^+$   $proB^+$   $proA^+$  episome and the comparable segment of the recipient chromosome can occur when the  $F^-$  possesses the  $proB-lac$  deletion, but not when the  $F^-$  possesses the  $proA-B$  deletion. The  $F'$   $lac^+$   $proB^+$   $proA^+$  episome contains an inverted  $lac$  operon, and therefore this  $F'$  can cause chromosome mobilization and transfer in both clockwise and counterclockwise directions (5). The data in Table 10 reveal that chromosome mobilization and transfer in the counterclockwise direction are reduced when the  $F^-$  possesses either the  $proB-lac$  or  $proA-B$  deletion mutation (matings 7 and 8, respectively). Also, the inversion within the  $F'$   $lac^+$   $proB^+$   $proA^+$  episome reduces the frequency of  $leu^+$  and  $arg^+$  recombinants which arise as a consequence of chromosome mobilization, even when the  $F^-$  recipients have no deletion mutations (matings 1 and 2 versus matings 5 and 6, Table 10). Based on the results presented in Fig. 8 and 9 and in Tables 9 and 10, we conclude that effective homologous pairing between the recipient chromosome and episome is not necessary for  $F$  and  $F'$   $lac^+$  transfer, but is necessary for the transfer of the  $F'$   $lac^+$   $proB^+$   $proA^+$  episome and for chromosome mobilization and transfer mediated by  $F$  and by  $F'$  episomes.

TABLE 10. *F'* and chromosome transfer by partially diploid *F'* donors to *F*<sup>-</sup> strains having deletion or point mutations<sup>a</sup>

Mating	Inheritance of <i>lac</i> <sup>+</sup> allele (%)		Inheritance of <i>pro</i> <sup>+</sup> allele(s) (%)		Inheritance of <i>leu</i> <sup>+</sup> allele (%)		Inheritance of <i>arg</i> <sup>+</sup> allele (%)	
	Actual	Relative to <i>F'</i> transfer	Actual	Relative to <i>F'</i> transfer	Actual	Relative to <i>F'</i> transfer	Actual	Relative to <i>F'</i> transfer
x 647 <i>F'</i> <i>lac</i> <sup>+</sup> <i>lacY</i> <sup>-</sup> X								
(1) x 708 <i>F</i> <sup>-</sup> <i>lacZ</i> <sup>-</sup> <i>proB</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	206	100	4.1	2.0	2.7	1.3	0.11	0.053
(2) x 710 <i>F</i> <sup>-</sup> <i>proA</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	—	—	5.4	2.0 <sup>b</sup>	2.9	1.1 <sup>b</sup>	0.23	0.086 <sup>b</sup>
(3) x 784 <i>F</i> <sup>-</sup> <i>proB-lac</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	328	100	0.82	0.25	0.71	0.22	0.084	0.026
(4) x 711 <i>F</i> <sup>-</sup> <i>proA-B</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	—	—	0.097	0.036 <sup>b</sup>	0.032	0.012 <sup>b</sup>	0.0026	0.00097 <sup>b</sup>
x 646 <i>F'</i> <i>lac</i> <sup>+</sup> <i>proB</i> <sup>+</sup> <i>proA</i> <sup>+</sup> / <i>lacY</i> <sup>-</sup> X								
(5) x 708 <i>F</i> <sup>-</sup> <i>lacZ</i> <sup>-</sup> <i>proB</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	160	98	167	102	0.24	0.15	0.011	0.0067
(6) x 710 <i>F</i> <sup>-</sup> <i>proA</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	—	—	119	100	0.52	0.44	0.018	0.015
(7) x 784 <i>F</i> <sup>-</sup> <i>proB-lac</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	256	102	247	98	0.10	0.040	0.013	0.0052
(8) x 711 <i>F</i> <sup>-</sup> <i>proA-B</i> <sup>-</sup> <i>leu</i> <sup>-</sup> <i>arg</i> <sup>-</sup> . . . . .	—	—	2.1	1.2 <sup>c</sup>	0.0039	0.0022 <sup>c</sup>	<0.0008	<0.00045 <sup>c</sup>

<sup>a</sup> All matings were of 40-min duration and were conducted as described in the legend to Fig. 9.

<sup>b</sup> Calculated by using the mean per cent transfer of *F'* *lac*<sup>+</sup> to x 708 and x 784 (267%).

<sup>c</sup> Calculated by using the mean per cent transfer of *F'* *lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> to x 708, x 710, and x 784 (178%).

The major difference between F and F' *lac*<sup>+</sup> on the one hand and the F' *lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> episome on the other is their size. By using genetic data and the molecular weights of F (31) and of the chromosome (13), we calculate that F, F' *lac*<sup>+</sup>, and F' *lac*<sup>+</sup> *proB*<sup>+</sup> *proA*<sup>+</sup> contain 1.7, 2.8, and 5.5% as much DNA as the chromosome, respectively. If effective homologous pairing between the lead region of a donor chromosome and the comparable portion of the recipient chromosome is required to achieve a substantial frequency of transfer, then it follows that a mechanism must exist to permit the transfer of the lead region of the donor chromosome into the recipient cell. We therefore suggest that all types of donors, be they F<sup>+</sup>, F', or Hfr, are capable of forcibly transferring an average of about 3% of their genetic material into F<sup>-</sup> recipients without the need for effective homologous pairing. Further genetic transfer, however, would require effective homologous pairing. Thus, the lack of genetic homology between episome and chromosome would not alter the kinetics of F and F' *lac*<sup>+</sup> transfer, since these elements are small enough to be forcibly transferred *in toto* by the donor cells. For genetic transfer by Hfr donors (and donors of long F' factors), it is probable that the amount of donor chromosome forcibly transferred by each donor cell would vary and that the mean amount of material transferred in this manner would increase in proportion to the length of mating time. In the experiments presented with Hfr OR1 (Fig. 6A, B, and C) and with F' ORF-206 (Fig. 9), about 1% of the donor cells were able to transfer a sufficient amount of their chromosomes so that effective homologous pairing with the segment beyond the *proA-B*<sup>-</sup> deletion could occur.

A corollary prediction of the above hypothesis on F' transfer is that there should be an observable difference in recombinant types issuing from matings between F<sup>-</sup> strains and donors with either short or long F' episomes. If short F' episomes are pushed into the recipient without the need for effective homologous pairing, then most recombinants (ca. 99%) should be heterozygous and partially diploid. This is precisely the result found for the recombinants formed in the mating with the F' *lac*<sup>+</sup> donor described in Fig. 8A. If the lead region of long F' episomes must undergo effective homologous pairing with the comparable segment of the recipient chromosome, then some recombinants (ca. 20 to 50%) for lead markers from F' will be haploid, and some recombinants (ca. 5 to 20%) having received the entire F' will be homozygous at some loci and partially diploid. Results of this type have been obtained in matings with F' ORF-1, which has

an F' containing 8% of the chromosome (5; *unpublished data*). The presence of the inverted *lac* operon in F' ORF-206 probably inhibits haploid recombinant formation for proximally transferred episome markers, although the frequency of haploid recombinants (5%, *see legend to Fig. 9*) is intermediate between the frequencies found in matings with donors of F' *lac*<sup>+</sup> and F' ORF-1.

On the basis of the results obtained in the experiments described in this section, we concluded that: (i) all donor strains, be they F<sup>+</sup>, F', or Hfr, are capable of forcibly transferring several per cent of their genetic material into F<sup>-</sup> recipients without a requirement for effective homologous pairing, and (ii) further transfer of the donor genome requires that the lead region of the donor chromosome forcibly transferred be effectively paired with the comparable region of the recipient chromosome. In view of the results obtained by us and by Pittard and Walker (47), we also believe that the effective homologous pairing required for chromosome transfer is also intimately involved in the first steps leading to haploid recombinant production.

#### SUMMARY DISCUSSION

In the first section of the RESULTS, the experiments conducted led us to conclude that both the Hfr and F<sup>-</sup> parent require a metabolizable energy source during conjugation and to suggest that the Hfr parent requires energy to initiate chromosome transfer and that the F<sup>-</sup> parent requires energy to control the rate of chromosome transfer.

In the second section of the RESULTS, the data obtained led us to suggest: (i) that DNA synthesis in the Hfr parent is required at the beginning of mating to initiate chromosome transfer and (ii) that, although continual DNA synthesis may normally accompany chromosome transfer, it is not necessary for transfer and therefore does not control the rate of chromosome transfer.

The experimental data in the third section of the RESULTS indicated that amino acid starvation of the Hfr parent led to a loss of donor ability, most of which was not immediately recovered when the amino acid was restored to the culture. This nonrecoverable loss in donor ability was interpreted as being due to loss of F pili (Curtiss and Caro; Bacteriol. Proc., p. 27, 1966; *manuscript in preparation*) with a resultant loss in specific pair-forming ability and to the loss of a postulated donor cell surface component needed to establish effective pairs with F<sup>-</sup> cells. The restorable loss of donor ability during amino acid starvation was interpreted to indicate a requirement for protein synthesis in the Hfr at the inception of mating for

initiation of chromosome transfer (29). Amino acid starvation of the  $F^-$  recipient, which permits completion of vegetative chromosome replication (42, 46), alters the apparent kinetics of chromosome transfer; this observation was interpreted to mean either that degradation of the donor chromosomal region last transferred at the time of mating interruption is not degraded in amino acid-starved  $F^-$  cells or that vegetative chromosome replication must reach a specific stage (e.g., completion) before the  $F^-$  can actively participate in the transfer of the donor chromosome with an expenditure of energy. The latter interpretation also implies that vegetative replication of the recipient chromosome and chromosome transfer may be mutually exclusive processes.

In the last section of the RESULTS, the data obtained led us to conclude (i) that  $F^+$ ,  $F'$ , and Hfr donors are capable of forcibly transferring several per cent of their genetic material into  $F^-$  recipients without a requirement for effective homologous pairing between the donor material transferred and the comparable segment of the recipient chromosome, and (ii) that more extensive transfer of the donor genome first requires that the forcibly transferred lead region of the donor chromosome undergo effective homologous pairing with the comparable region of the recipient chromosome. We further suggested that the effective homologous pairing required for chromosome transfer is also intimately associated with the initial events leading to haploid recombinant formation.

The conclusions and suggested interpretations of the data presented in this manuscript, along with the results of studies conducted in other laboratories, can be used to construct a conceptual model for the steps during bacterial conjugation between Hfr and  $F^-$  bacteria. With regard to specific pair formation, the work of Brinton and associates (9-11) and our work (Curtiss and Caro, *Bacteriol. Proc.*, p. 27, 1966; *manuscript in preparation*) have shown an obligate requirement for the presence of F pili on the donor cell surface. We (22) have also shown that specific pair formation occurs in the absence of active energy metabolism in both parents. The conversion of a specific pair, which is experimentally defined as a union stable to dilution (26) to an effective pair, which is operationally defined as a union ready to initiate chromosome transfer, is presently a poorly understood process. We have suggested, from results obtained in matings with the DNA-less minicells (14, 16), from the isolation and characterization of mutant donor strains (Curtiss and Stallions, *unpublished data*), and from our results in experiments on amino acid starvation of donor strains, that

donor cells may possess a surface component in addition to F pili which is necessary for promoting effective unions between donor and recipient cells and for preventing matings between donor cells. The establishment of effective cell contact between a donor and recipient cell would then act as a stimulus to the donor cell to initiate chromosome mobilization, as suggested by Jacob, Brenner, and Cuzin (38).

Chromosome mobilization, which is operationally defined as the production of a donor chromosome being ready for linear sequential transfer to a recipient cell, would occur in the Hfr cell and be under the genetic control of F (38). There would be an initial requirement for protein synthesis (29, 50; third section of RESULTS) to allow for the synthesis of an F-coded initiator (24, 38) followed by an obligate requirement for DNA synthesis (4, 16, 36, 50; second section of RESULTS). Both of these synthetic activities would require active energy metabolism in the Hfr parent at the inception of mating (first section of RESULTS). This DNA synthesis in the Hfr parent would act as the driving force for the initiation of chromosome transfer, with the introduction of several per cent of the leading extremity of the donor chromosome into the recipient parent (second and fourth sections of RESULTS). More extensive chromosome transfer would then require the establishment of effective homologous pairing between the lead region of the donor chromosome and the comparable segment of the recipient chromosome (fourth section of RESULTS). The  $F^-$  parent would then wind in the donor chromosome with an expenditure of energy (20; first section of RESULTS). This process would ensure that homologous regions of the donor and recipient chromosomes would be brought into synaptic union to provide for the known orderly and efficient integration of donor genetic material into recombinants.

The above-described events and parental activities during bacterial conjugation are supported by many experimental observations, and we are not aware of any findings which would appreciably alter the model as proposed. Certain other aspects of bacterial conjugation, especially those concerned with the mechanism of chromosome transfer, are not yet resolved, since contradicting results and interpretations of data do exist. The dependency of chromosome transfer on continual DNA synthesis in the Hfr parent as suggested by Jacob, Brenner, and Cuzin (38) is not supported by our findings or by those of Pritchard (48). On the other hand, there is evidence which has been interpreted to indicate that replication of the donor chromosome normally accompanies transfer and that this replication occurs in the Hfr

parent (6, 33, 34, 49). Philosophically, we might wish that what normally occurs would indicate what must of necessity occur. However, we believe that chromosome transfer is usually a cooperative effort by both mating partners and that alternative means for accomplishing the same end result might have been developed during the course of bacterial evolution. Our finding that the  $F^-$  parent is responsible, at least in part, for controlling the rate of chromosome transfer would seem to eliminate the requirement that the donor-mediated replication of its chromosome during transfer controlled the rate of transfer as first suggested by Jacob, Brenner, and Cuzin (38). Certainly, replication of the DNA strand retained in the Hfr cell during chromosome transfer would ensure the genetic viability of the donor cell, since in the absence of DNA synthesis mating would often be a suicidal act for the Hfr parent.

There also exist in the literature and in this paper conflicting data and contradictory conclusions on metabolic activities occurring in the  $F^-$  parent during chromosome transfer. We have demonstrated an association between energy metabolism in the  $F^-$  parent and control of the rate of chromosome transfer and a requirement for effective homologous pairing between the lead region of the Hfr chromosome and the comparable segment of the recipient chromosome to achieve a substantial amount of chromosome transfer. It would therefore appear that the most plausible explanation for the results obtained with amino acid-starved  $F^-$  strains would be that completion of vegetative chromosome replication must occur in the  $F^-$  prior to the winding in of the donor chromosome. A corollary suggestion would be that chromosome transfer and vegetative replication of the recipient chromosome might be mutually exclusive processes. A prediction of the above hypothesis is that prevention of DNA synthesis in a logarithmically growing  $F^-$  population should markedly reduce the number of  $F^-$  cells capable of involving themselves in chromosome transfer. While Bonhoeffer's (7) results are in complete accord with this prediction, the results of thymine starvation of *thy<sup>-</sup>*  $F^-$  strains are not (48; second section of RESULTS). Further experimentation is now in progress in the hope of resolving these problems and to test the hypothesis that completion of vegetative chromosome replication in the  $F^-$  is necessary for chromosome transfer.

[Note added in proof. During this past summer, we (Curtiss and Williams, unpublished data) conducted a series of experiments on the effects of amino acid starvation on the  $F^-$  parent. The results obtained strongly suggest that chromo-

some transfer is not dependent upon completion of an old round or initiation of a new round of vegetative replication of the  $F^-$  chromosome. The cause of the effects described in the third section of the Results is as yet unknown.]

Another problem associated with metabolic activities occurring in the  $F^-$  parent during conjugation concerns the effects of purine starvation of *pur<sup>-</sup>*  $F^-$  strains. Freifelder (30) demonstrated that purine starvation depressed the frequency of episome and chromosome transfer, whereas Gross and Caro (34) observed no effect of purine starvation or UV irradiation of the  $F^-$  parent on chromosome transfer. We (Curtiss and Mays, unpublished data) have partially confirmed Freifelder's results, but have also noted that purine starvation inhibits certain events involved in the integration of transferred donor genetic material. We also suggest the possibility that certain treatments of the  $F^-$  parent, such as purine starvation, might involve restriction of normally transferred donor genetic material. Gross and Caro (34) used *E. coli* C as their  $F^-$  recipient, and this strain is normally incapable of restriction.

The conclusions drawn and the suggestions made on the basis of the data presented in this manuscript have been substantiated in part by the studies of Cohen et al. (15, 16) on the physical properties of DNA transferred by  $F^+$ ,  $F'$ , and Hfr donors to the DNA-less minicells isolated and described by Adler et al. (3). This research has also permitted a more precise definition of certain steps and features of the conjugal transfer of genetic information from donor to recipient strains of *E. coli* K-12.

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